JUL 2 0 1945

Volume 35 *Number 7*

Phytopathology

AN INTERNATIONAL JOURNAL

July 1945

Official Organ of

The American Phytopathological Society

$6.25 per year, Canada $6.00 per year, United States and dependencies $6.50 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter.

TABLE OF CONTENTS

Peter Henry Rolfs ..... Howard S. Fawcett 491

Studies in the Fusarium damping-off of conifers. III. Relation of temperature and sunlight to the pathogenicity of Fusarium. Howard Tint 498

An apparatus for the growth of plants under controlled temperature levels ..... Howard Tint 511

A preliminary report on further studies of physiologic specialization in Ustilago hordei ..... T. F. Yu and C. T. Fang 517

Application of penicillin to crown gall. J. G. Brown and Alice M. Boyle 521

The mode of infection and the incubation period in the stem smut of grasses, Ustilago spegazzinii (U. hypodytes). George W. Fischer 525

The reaction of 21 species in the Cucurbitaceae to artificial infection with cantaloupe powdery mildew (Erysiphe cichoracearum D C.) ..... Thomas W. Whitaker and Dean E. Pryor 533

Technique for hastening foliage symptoms of psorosis of citrus. James M. Wallace 535

The invasion of the internal structure of cotton seed by certain Fusaria ..... B. A. Rudolph and G. J. Harrison 542

The field reactions of certain oats hybrids and varieties to stem rust. M. B. Moore, H. K. Hayes, and E. C. Stakman 549

Nigrospora oryzae (B. and BR.) Petch on maize ..... J. H. Standen 552

Athracnose of garden pea ..... S. H. Ou and J. C. Walker 565

Phytopathological Notes 571

Physiologic specialization in Helminthosporium gramineum Rabh. ..... D. C. Arny

Nematode infection of Croft Easter lilies ..... Wilbur D. Courtney

Inoculations of the evergreen species of Prunus (Laurocerasus) with Tranzschelia pruni-spinosae ..... Clayton O. Smith

Polyporus versicolor on Asiatic chestnut. Ray R. Hirt and J. L. Lowe

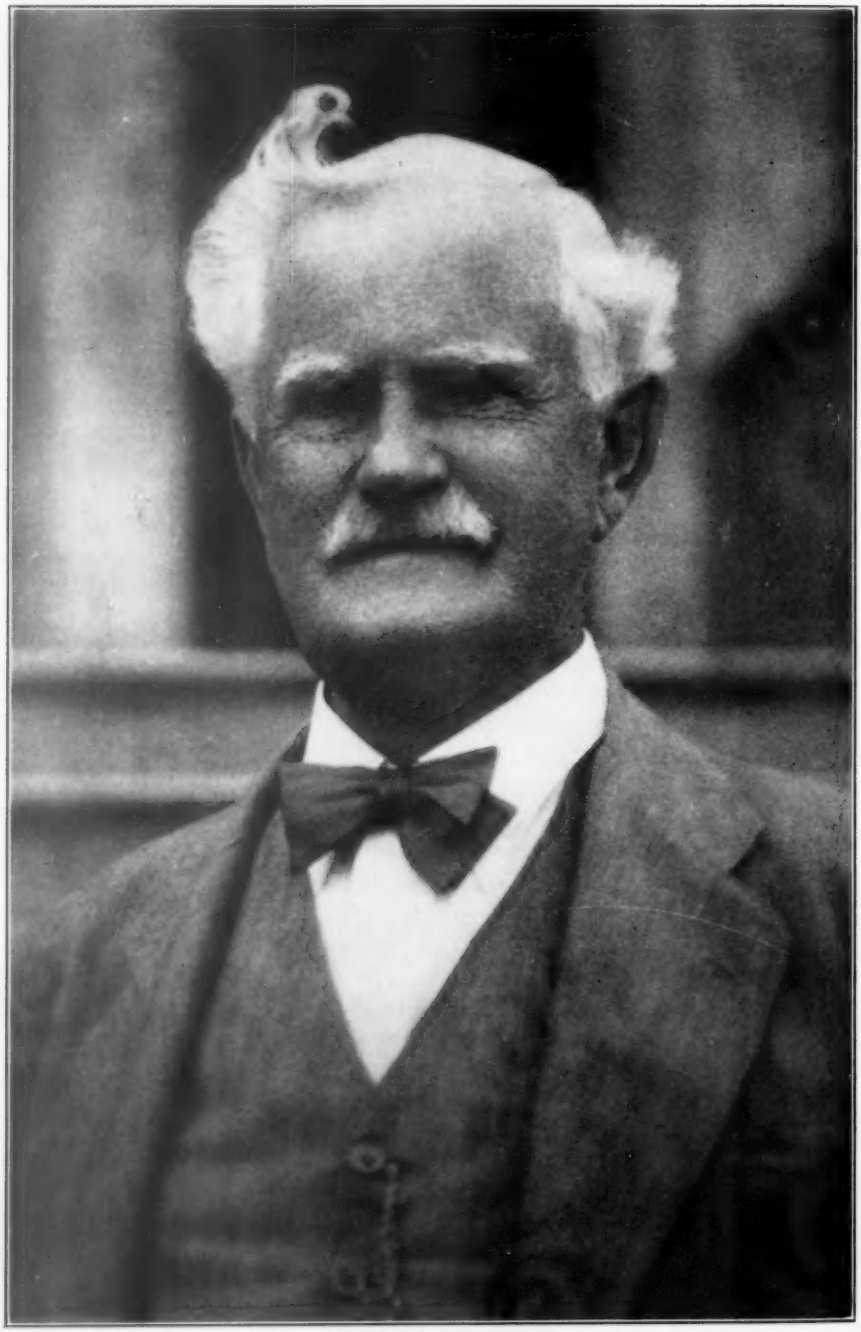
PETER HENRY ROLFS
1865–1944

HOWARD S. FAWCETT

Peter Henry Rolfs, who was among the pioneer plant pathologists of America, and a charter member of the American Phytopathological Society, came to the evening of a life of great usefulness to botanical and agricultural science at Gainesville, Florida, February 23, 1944. He was born on April 17, 1865, at LeClaire, Iowa. A course of study at the Iowa State College at Ames followed the earlier years on his father's Iowa farm. His B.Sc. degree at Iowa State College was obtained in 1889 and his M.S. in 1891. The University of Florida honored him with a Doctor of Science degree in 1920.

Following his studies at Iowa State College, he became Professor of Natural Science at the Florida Agricultural College in Lake City from 1891 to 1899. At Clemson College, South Carolina, he was botanist and bacteriologist from 1899 to 1901. In 1901 he was officially known as Plant Pathologist in accepting a position with the United States Department of Agriculture at the Sub-tropical Plant Introduction Garden, Miami, Florida. He seems to have been the first to successfully bud and graft avocados. He was appointed to the Directorship of the Florida Agricultural Experiment Station at the University of Florida, at Gainesville, in 1906, where he remained until 1921. It was during the first years of his directorship (1906 to 1912) that I learned to know and appreciate his personality and worth and to receive the benefit of his experience, enthusiasm, and his helpful, kindly advice in carrying on research in the then newly differentiated field of plant pathology. I was, I believe, his first graduate student. Added responsibility came later to Professor Rolfs in being appointed Director of the Agricultural Extension Division in 1913 and Dean of the Agricultural College of the University of Florida in 1915.

When a request came to the United States Department of State from Brazil for a man best fitted to found a new agricultural college along practical applicational lines, Dr. Rolfs was chosen. In 1921 he went to Brazil and began the development on a new site in the State of Minas Geraes at Viçosa, the institution now known as Escola Superior de Agricultura. This institution, the building of which he supervised with great pains and attention to details from the ground up, is a real monument to Dr. Rolfs' memory. At the same time, it is one of the most valuable and practical contributions to international good will. The esteem with which he was held after his retirement in Brazil in 1933 is shown by an effort to highly praise one of his successors in saying "this man gives promise of being a second Rolfs." Another indication is that, in 1943, a bronze bust of Dr. Rolfs was placed at the front entrance of the main college building at Viçosa.



PETER HENRY ROLFS
1865–1944

The lasting influence of his work in Brazil is indicated by the fact that hundreds of agriculturists, not only from Minas Geraes, but from other states of Brazil, attend a "Farmers Week" at the college. The attendance at Farmers Week passed the 900 mark in 1944. The regular college attendance in 1943 was 1200. This is, no doubt, having far reaching influence among the solid citizenry of Brazil. No small part of this work by Professor Rolfs in Brazil is to be attributed to the help of his daughter, Clarissa Rolfs, who was constantly with him and became even more proficient in the Portuguese language than he. With her he shared his agricultural interests and published 20 or more papers jointly.

He was made "Consultor de Agricultura" in the State of Minas Geraes from 1928 to 1933, the time of his retirement. In 1892 Dr. Rolfs married Miss Effie Stone who died in 1929, leaving two daughters and four grandchildren. He stayed in Brazil for several years after his retirement with one of his daughters, Miss Clarissa Rolfs.

He was a member of the following scientific societies and organizations: Emeritus Life Member, American Association for the Advancement of Science, 1938; charter member of Botanical Society of America, and of The American Phytopathological Society; Honorary Life Member, Florida State Horticultural Society, 1920; President, 1907–8, Chairman Executive Committee, 1908–21; Member International Association of Botanists; St. Louis Academy of Science; Iowa Academy of Science; Association for Promotion of Horticultural Science; American Pomological Society; Associate Member, American Association of Economic Entomologists; State Chairman, Council of National Defense, 1917–18; and member of Alpha Zeta, Phi Kappa Phi, and Sigma Xi.

That Professor Rolfs was one of the pioneers in mycology and plant pathology is shown by some of his publications. In "A fungus disease of the San Jose scale" in 1897, he opened up the subject of fungi as part of the biological control of insects in Florida which paved the way for later investigations in this field. He gave attention early in Florida to diseases of economic crops: tomatoes 1898, 1907; oranges 1898, 1904, 1906, 1908. He carried his interest in plant pathology to Brazil where, although his main interest was horticulture, he yet found time to give some attention to diseases. He established at the institution in Viçosa a department for teaching and research in plant pathology, headed by a plant pathologist from the United States, A. S. Müller. The breadth of his interests is shown in his papers ranging from "Lichens in Florida" for the St. Louis Academy of Science to "Sub-tropical vegetable growing" in his book published by Macmillan Company and "Domesticating anti-lepric species in Brazil" in the Leprosy Review.

The scope of his interests in plant pathology, as well as other agricultural fields, may be gained by the long list of his publications:

The seed coats of Malvaceae. Bot. Gaz. **17**: 33–39. 1892.
Insecticides and fungicides. Fla. Agr. Exp. Stat. Bull. 23. 1893.

The tomato and some of its diseases. Fla. Agr. Exp. Stat. Bull. 21. 1893.
Insects and insecticides. Proc. Fla. State Hort. Soc. **8**: 80–84. 1895.
The San Jose scale. Fla. Agr. Exp. Stat. Bull. 29. 1895.
Some market vegetables for Florida. Fla. Agr. Exp. Stat. Bull. 31. 1895.
Advancement made in insecticides. Proc. Fla. State Hort. Soc. **9**: 101–102. 1896.
The brief study in insect dissemination. Proc. Fla. State Hort. Soc. **9**: 99–100. 1896.
Injurious insects of the year. Proc. Fla. State Hort. Soc. **9**: 96–99. 1896.
Vegetable growing in the South for northern markets. Southern Planter Pub. Co. 1896.
The visit to the Gypsy Moth Commission. Proc. Fla. State Hort. Soc. **9**: 102–105. 1896.
An act for the control and extirpation of diseases and insects of the agricultural products of the state of Florida. Proc. Fla. State Hort. Soc. **10**: 111–116. 1897.
A fungus disease of the San Jose scale (*Sphaerostilbe coccophila* Tul.). Fla. Agr. Exp. Stat. Bull. 41. 1897.
Injurious insects of the year. Proc. Fla. State Hort. Soc. **10**: 94–97. 1897.
Insect diseases. Proc. Fla. State Hort. Soc. **10**: 97–104. 1897.
Diseases of the tomato. Fla. Agr. Exp. Stat. Bull. 47. 1898.
Injurious insects and diseases of the year. Proc. Fla. State Hort. Soc. **11**: 80–93. 1898.
Orange diseases and insects. Proc. Fla. State Hort. Soc. **11**: 34–43. 1898.
Strawberries and miscellaneous fruits. Proc. Fla. State Hort. Soc. **11**: 95–99. 1898.
Pineapple fertilizers. Fla. Agr. Exp. Stat. Bull. 50. 1899.
Pineapple fertilizers. Proc. Fla. State Hort. Soc. **12**: 90–96. 1899.
Pineapple growing. U. S. Dept. Agr. Farmers' Bull. **140**: 1–47. 1901.
Florida lichens. Trans. St. Louis Acad. Sci. **11**: 25–39. 1902.
The Tropical Laboratory. Proc. Fla. State Hort. Soc. **15**: 49–52. 1902.
Probable results of draining the Everglades. Proc. Fla. State Hort. Soc. **16**: 46–51. 1903.
The avocado in Florida; its propagation, cultivation, and marketing. U. S. Dept. Agr. Bur. Plant Indus. Bull. **61**. 1904.
Citrus diseases. Proc. Fla. State Hort. Soc. **17**: 89. 1904.
Relation of science to horticulture. Proc. Fla. State Hort. Soc. **17**: 57–66. 1904.
Wither-tip and other diseases of citrus trees and fruits caused by *Colletotrichum gloeosporioides*. U. S. Dept. Agr. Bur. Plant Indus. Bull. **52**. 1904.
Citrus fruit growing in the Gulf States. U. S. Dept. Agr. Farmers' Bull. **238**. 1906.
The Experiment Station Co-operative work. Proc. Fla. State Hort. Soc. **19**: 41–45. 1906.
Fall dropping of citrus fruit. Fla. Agr. Exp. Stat. Press Bull. 60. 1906.
Mangoes. Proc. Fla. State Hort. Soc. **19**: 65. 1906.
New opportunities in subtropical fruit growing. U. S. Dept. Agr. Yearbook **1905**: 439–454. 1906.
Alfalfa. Fla. Agr. Exp. Stat. Press Bull. 66. 1907.
Okra, or gumbo (*Hibiscus esculentus*.) *In:* Bailey, L. H., Cyclopedia of American Horticulture, 6th ed., vol. 3, p. 1125. 1907. The Macmillan Co., New York.
Onion culture in the south. *In:* Bailey, L. H., Cyclopedia of Horticulture, 6th ed., vol. 3, pp. 1139–1140. 1907. The Macmillan Co., New York.
Pineapple. *In:* Bailey, L. H., Cyclopedia of Horticulture, 6th ed., vol. 3, pp. 1343–1345. 1907. The Macmillan Co., New York.
President's annual address. Proc. Fla. State Hort. Soc. **20**: 21–26. 1907.
Tomato diseases. Fla. Agr. Exp. Stat. Bull. 91. 1907.
Citrus bloom dropping. Fla. Agr. Exp. Stat. Press Bull. 84. 1908.
Citrus foot rot. Fla. Agr. Exp. Stat. Press Bull. 96. 1908.
Fungus diseases of scale insects and whitefly. (With H. S. FAWCETT.) Fla. Agr. Exp. Stat. Bull. 94. 1908.
President's annual address. Proc. Fla. State Hort. Soc. **21**: 30–34. 1908.
Corn. Fla. Agr. Exp. Stat. Bull. 100. 1909.
Eggplant. *In:* Bailey, L. H., Cyclopedia of American Horticulture, 6th ed., vol. 2, pp. 521–524. 1909. The Macmillan Co., New York.
Florida horticulture. *In:* Bailey, L. H., Cyclopedia of American Horticulture, 6th ed., vol. 2, pp. 590–592. 1909. The Macmillan Co., New York.
Russeting of citrus fruits. Fla. Agr. Exp. Stat. Press Bull. 117. 1909.
Seed corn. Fla. Agr. Exp. Stat. Press Bull. 136. 1909.
What the Experiment Station is doing for the fruit and vegetable grower. Proc. Fla. State Hort. Soc. **22**: 27–30. 1909.
Hay. Fla. Agr. Exp. Stat. Press Bull. 153. 1910.
Horticulture and agricultural education in Florida. Proc. Fla. State Hort. Soc. **23**: 26–35. 1910.
Plant-bugs in orange groves. Fla. Agr. Exp. Stat. Press Bull. 155. 1910.
The valuation of carbohydrates and protein in commercial feeds. Proc. Soc. Prom. Agr. Sci. **31**: 107–109. 1910.

The avocado in Florida, its propagation, cultivation, and marketing. Fla. Grower 5(5): 3. 1911.
Diseases of citrus fruits. (With H. S. FAWCETT and B. F. FLOYD.) Fla. Agr. Exp. Stat. Bull. 108. 1911.
Japanese cane—fertilizer experiments. Fla. Agr. Exp. Stat. Press Bull. 167. 1911.
The mango in Florida. Proc. Amer. Pomol. Soc. 32: 34–43. 1911.
The withertip disease in Florida. Pomona College Jour. Econ. Bot. 1: 107–108. 1911.
Notes on citrus investigations in Florida. Proc. Soc. Prom. Agr. Sci. 33: 29–36. 1913.
Culture, fertilization, and frost protection of citrus groves in the Gulf States. U. S. Dept. Agr. Farmers' Bull. 542. 1913.
Discussion on tropical fruits. Proc. Fla. State Hort. Soc. 26: 185–191. 1913.
Fungus diseases of scale insects and whitefly. (With H. S. FAWCETT.) Fla. Agr. Exp. Stat. Bull. 119. 1913.
Propagation of citrus trees in the Gulf States. U. S. Dept. Agr. Farmers' Bull. 539. 1913.
Sites, soils, and varieties for citrus groves in the Gulf States. U. S. Dept. Agr. Farmers' Bull. 538. 1913.
Tomato diseases. Fla. Agr. Exp. Stat. Bull. 117. 1913.
E. O. Painter and Florida horticulture—a quarter century of progress in Florida. Proc. Fla. State Hort. Soc. 27: 214–231. 1914.
Materials for correcting soil acidity. Fla. Agr. Exp. Stat. Press Bull. 227. 1914.
Using ground limestone. Fla. Agr. Exp. Stat. Press Bull. 228. 1914.
Mangoes in Florida. Fla. Agr. Exp. Stat. Bull. 127. 1915.
Avocado culture. Fla Agr. Exp. Stat. Press. Bull. 244. 1916.
Avocado propagation. Calif. Citrog. 1(8): 31. 1916.
Avocado propagation. Fla. Agr. Exp. Stat. Press Bull. 243. 1916.
Guava propagation. Fla. Agr. Exp. Stat. Press Bull. 246. 1916.
Present need of Florida horticulture. Proc. Fla. State Hort. Soc. 29: 15–18. 1916.
Roselle mildew. Fla. Agr. Exp. Stat. Press Bull. 255. 1916.
Subtropical vegetable gardening. 309 pp. 1916. The Macmillan Co., New York.
Care of trees after freezing temperatures. Fla. Agr. Exp. Stat. Press Bull. 263. 1917.
Castor beans. Fla. Univ. Agr. Ext. Cir. 2. 1917.
Citrus trees damaged by cold. Fla. Agr. Exp. Stat. Press Bull. 262. 1917.
Our nation is calling for more hogs at high prices. Fla. Univ. Agr. Ext. Cir. 1. 1917.
Rhodes grass. Fla. Agr. Exp. Stat. Bull. 138. 1917.
Council of state defense. Proc. Fla. State Hort. Soc. 31: 141–147. 1918.
The Experiment Station. Proc. Fla. State Hort. Soc. 33: 174–179. 1920.
Fertilizers for pineapples. Fla. Agr. Exp. Stat. Press Bull. 321. 1920.
Live stock in Minas Geraes. Brasilian Bus., July, 1921.
Brasil's fruit opportunity. Brasilian Bus., Oct., 1921.
Cattle in Brazil. Farmer and Stockman, June 15, 1922.
Citrus in Brazil. Fla. Grower 25(25): 5. 1922.
O Congresso Internacional de Algodão. O Agricultor, Nov., 1922.
Ensino agricola. Conferencia perante a Sociedade Nacional de Agricultura. 32 pp. Dec. 15, 1922.
Turning oranges into gold. Brasilian American, Jan. 6, 1923.
Transformando laranjas em ouro. Minas Geraes, Jan. 14–16, 1923. *Also in:* Lavoura e Criação, May, 1923. *Also in:* O Agricultor, May, 1923.
A. Alexia. Minas Geraes. Feb. 26–27, 1923. *Also in:* Chacaras e Quintaes, Apr., 1923.
The Market for Brasilian fruits. Brasilian Bus., Feb., 1923.
Relatorio Sobre a Exposição de Frutas e Flores na Exposição deo Centennario. Minas Geraes, March 12–13, 1923.
Cigarrinho de Canna de Assucar. Minas Geraes. March 20, 1923.
O Fome para o Algodão. Minas Geraes. Apr. 5, 1923.
Brazilian fruit and the centennial. Fla. State Hort. Soc. Proc. 36: 33–39. 1923. *Also in:* Fla. Grower 27(16): 4–5. 1923.
A Superioridade do Gado de Puro Sangue. O Agricultor, May, 1923. *Also in:* Rev. Agr. Mineira, Sept., 1923.
Cochonilha de Canna de Assucar. Minas Geraes, May 30, 1923.
The international cotton conference. Brasilian Bus., June, 1923.
O mercado para as frutas do Brasil. Brasil Ferro-Carril, Aug. 2–16, 1923. *Also in:* A Lavoura, June, 1923.
The second regional exposition at Lavras. Brasilian Bus., Oct., 1923.
A prevençãa de Destruiçã de Sementes por Insectos. Rev. Agr. Mineira, Oct., 1923. *Also in:* A Lavoura, Feb., 1924.
A Guerra Contra os Insectos. O Agricultor, Nov., 1923 and May, 1924.
Educação Agricolae a Economia Nacional. A Lavoura, Nov., 1923.
Preventing damage to stored grain. Brasilian Bus., Nov., 1923.

A Escola Superior de Agricultura e Veterinaria de Minas Geraes e um Systema Nacional de Ensino Agricola. Jornal do Commercio, Dec. 17, 1923. *Also in:* A Lavoura, Dec., 1923 and Jan., 1924. *Also in:* Rev. Agr. Mineira, Feb., 1923.

As Cuyabanas. Rev. Agr. Mineira, Feb., 1924.

A Chaulmoogra, Onde e Como se Deve Plantal-a. A Lavoura, March, 1924.

Extinecão da Tiririca. A Lavoura, March, 1924.

Experimentação com Adubos a Cultura da Batata Ingleza. Rev. Agr. Mineira, Apr., 1924.

A Magnificent Palm. Brasilian Bus., July, 1924.

Cultura Secca do Arroz. Cidade de Viçosa, July 19–26 and Aug. 2, 1924.

Praga dos Cafezaes. Minas Geraes. July 24, 1924.

Docença do Ficus. Minas Geraes, Aug. 3, 1924.

Algodão. Cidade de Viçosa, Aug. 9, 1924, and Oct. 11, 1924.

A Industria de Laticinios no Estado de Minas Geraes. Minas Geraes, Aug. 10, 1924.

Quarta Exposição Agro-Pecuaria de Lavras. Minas Geraes, Aug. 23, 1924.

Arroz Sem Irrigação. Rev. Agr. Mineira, Aug. 24, 1924. *Also in:* O Excelsior, July 20–27, 1924.

Rice without irrigation. Brasilian Bus., Aug., 1924.

A Semana do Milho. Cidade de Viçosa, Sept. 13, 1924.

Exposição de Aves. Cidade de Viçosa, Sept. 20, 1924.

Milho, Batata Doce e Arroz. Cidade de Viçosa, Sept. 27, 1924.

The Avocado. Brasilian Bus., Sept., 1924.

A Beringela. Cidade de Viçosa, Oct. 4, 1924.

O Abacate. A Lavoura, Oct.–Nov., 1924.

Ensino Agronomico. Cidade de Viçosa, Nov. 8, 1924.

Palha de Cafe Vale Ouro. Cidade de Viçosa, Nov. 15, 1924.

Mal-di-Gomma. Almanach de Chacaras e Quintaes, 1924. *Also in:* Rev. Agr. Mineira, Feb., 1924.

Review of Fawcett and Lee's "Citrus Diseases of the World." Chacaras e Quintaes **34**: 456. 1926.

Amaryllis Hybridos. Boletim da Imprensa No. 8. *Also in:* Bol. de Agr. Zootech. e Vet. Nos. 7–9, 1927.

Cuidados com as Mudas de Citrus. Boletim da Imprensa No. 9, 1927.

Estatutos da Escola Superior de Agricultura e Veterinaria do Estado de Minas Geraes, sede em Viçosa. Minas Geraes Sec. de Agr. Bol. 1, 18 pp. 1927.

Lista e Descripção das Variedades de Citrus. Boletim da Imprensa No. 10. 1927.

Mal-di-Gomma das Laranjeiras e Seu Tratamento. Chacaras e Quintaes **36**: 276–277. 1927.

Regulamento da Escola Superior de Agricultura e Veterinaria do Estado de Minas Geraes. Impr. Off. de Minas Geraes, Bello Horizonte, 56 pp. 1927.

Renda de Algumas Qualidades de Milho. Bol. de Agr. Zootech. e Vet. **1**(1): 17–19. 1928.

O Arroz, Sua Cultura em Vargens Altas. Bol. de Agr. Zootech. e Vet. **1**(2): 5–7. 1928.

Augmento Rapido na Producção de Milho. Bol. de Agr. Zootech. e Vet. **1**(3): 3–6. 1928.

Uma Cultura Desprezada. Bol. de Agr. Zootech. e Vet. **1**(3): 6–7. 1928.

Introducção ao Discurso aos Criadors Mineiros. Bol. de Agr. Zootech. e Vet. **1**(4/5): 101–106. 1928.

Canna de Cavallo para Pastagens Artificiaes. Bol. de Agr. Zootech. e Vet. **1**(8/9): 13–15. 1928.

Ensino agricola Mineiro e Brasileiro. Segunda Conferencia Nacional de Educação, Nov. 4–11, 1928. 31 pp.

Palha de Cafe como Adubo. Bol. da Imprensa No. 24. 1928.

Preparo de Calda Bordaleza. Bol. da Imprensa No. 22. 1928.

Veneno Contra Abelha Cachorra. Bol. da Imprensa No. 25. 1928.

Algumas Considerações Sobre o Ensino Agricola do Estado de Minas Geraes. (With C. ROLFS.) O Agricultor, **8**(1). 1929.

Fertilizantes de Fazendas, Sua Producção e Utilidade. Bol. de Agr. Zootech. e Vet. **2**(3): 3–11. 1929.

Boas Mudas, Base da Citricultura. (With C. ROLFS.) Bol. de Agr. Zootech. e Vet. **2**(4): 5–12. 1929.

Escola Superior de Agricultura e Veterinaria do Estado de Minas Geraes. (With C. ROLFS.) Bol. da União Pan-Americana, **31**: 364–378. 1929. Reprinted as Serie Sobre Agricultura, No. 26. 15 pp.)

La Escuela Superior de Agricultura e Veterinaria de Minas Geraes. (With C. ROLFS.) Bol. de la Union Panamericana **58**: 499–515. 1929. (Reprinted as Serie Sobre Agricultura, No. 53. 17 pp.)

Laranja Satsuma Owari. (With C. ROLFS.) Bol. de Agr. Zootech. e Vet. **2**(5): 17–18. 1929.

Progressive Minas. Her Modern Agricultural College. (With C. ROLFS.) Brasilian Bus., 1929.

Subtropical vegetable growing. 2d edition. 309 pp. The Macmillan Co., New York, 1929.
The Avocado. (With C. ROLFS.) Brasilian Bus., Feb., 1931. *Also in:* Brasilian American, Feb. 28, 1931.
Propagação do Citrus no Brasil. (With C. ROLFS.) Bol. da União Pan-Americana, **33**(4/5, 6, 7): 1931. (Reprinted as serie sobre Agricultura, Nos. 36–38. 64 pp.)
Canna Cavallo para Pastagens Artificiaes. Minas Geraes, May 30, 1931.
O Milho. Minas Geraes, May 31, 1931.
Consumo Baixa e Não Superproducção. Minas Geraes. July 5, 1931. *Also in:* Bol. de Agr. Zootech. e Vet. **4**(7/9): 3–4. 1931.
A Aclimação e Domesticação das Especies Anti-Lepricas. Conferencia perante a Sociedade Nacional de Agricultura. A Lavoura, July, Aug., and Sept., 1931. *Also in:* Minas Geraes, Oct 7, 1931. Resumes in Jor. de Commercio, May 24, 1931; *in* A Lavoura, June 15, 1931; *and in* O Noite, May 21, 1931.
Progresso do Ensino Agricola em Minas. Minas Geraes, Oct. 3, 1931.
Aproveitamento de Braços. Minas Geraes, Oct. 5–6, 1931. *Also in:* Bol. de Agr. Zootech. e Vet. **4**(7–9): 80–86. 1931.
As Maquinas Agricolas Inferiores São Anti-Economicas. Minas Geraes, Oct. 11, 1931.
Cada Agricultor Uma Unidade Importante. Minas Geraes, Oct. 12/18, 1931.
Aprender dos Outros. Minas Geraes, Oct. 14, 1931.
A Cultura Mecanica e os Braços. Minas Geraes, Oct. 15, 1931.
A Força Mecaniça e a Producção Media. Minas Geraes, Oct. 16, 1931.
Os Tinhorões ou Caladiuns. Minas Geraes, Nov. 7, 1931. *Also in:* Bol. de Agr. Zootech. e Vet. **5**(1): 47–49. 1932.
O Abacateiro; Inicio Racional da Sua Exploração. (With C. ROLFS.) Jor. de Commercio, Dec. 23/24, 1931.
Palha de Café como Adubo. Minas Geraes, Dec. 30, 1931.
Augmento rapido na producção do milho. Minas Geraes Sec. de Agr. Serie Agr. do Serviço de Publicidade No. 4, 5 pp. 1931.
A cultura da sapucainha. (With C. ROLFS.) Bol. de Agr. Zootech. e Vet. **4**(10–12): 5–28. 1931.
A muda de citrus; pedra angular da industria citricola. (With C. ROLFS.) Oficinas Graphicas da Estatistica, Bello Horizonte, 126 pp. 1931.
Escola Superior de Agr. e Vet. Primeiro annuario, 1927. Impr. Off. de Minas Geraes, Bello Horizonte, 111 pp. 1931.
Introducção de "A Muda de Citrus." (With C. ROLFS.) Bol. de Agr. Zootech. e Vet. **4**(4/6): 3–9. 1931.
O Abacate; Variedades Enxertadas e Sue Plantio no Pomar. (With C. ROLFS.) Bol. de Agr. Zootech. e Vet. **4**(7/8/9): 5–12. 1931.
O ensino agricola no grau medio e sua organização no Brasil. *In:* A Escola Regional, published by the Associação Brasileira de Educação, pp. 66–92. 1931.
Propagación de Frutas Citricas en el Brasil. (With C. ROLFS.) Boletin de la Unión Panamericana. **65**: 469–491, 603–627, 735–753. 1931. Reprinted as Serie sobre Agricultura, Nos. 72–74. 65 pp.)
Fertilizantes de Refugos. (With C. ROLFS.) Bol. de Agr. Zootech. e Vet. **5**(1): 61–69. 1932.
Porque Alguns Abacateiros não Fruitificam. Chacaras e Quintaes. **45**: 88. 1932.
Poda das Raizes do Transplantio de laranjeira. Chacaras e Quintaes. **45**: 203–204. 1932.
Porque o Abacateiro não Produz. (With C. ROLFS.) Chacaras e Quintaes. **45**: 203–207. 1932.
Viagem de Estudos ao Estado de São Paulo. Bol. de Agr. Zootech. e Vet. **4**(4): 47–55. 1932.
Esterilidade do Abacateiro. (With C. ROLFS.) Bol. de Agr. Zootech. e Vet. **5**(5): 35–36. 1932.
The Avocado, How to insure productiveness. (With C. ROLFS.) Brasilian Bus. **12**(6). 1932.
Palha de Café e Cinzas da Mesma como Adubo. Bol. de Agr. Zootech. e Vet. **5**(8): 142–147. 1932.
Rejuvenescimento de Arvores de Citrus. (With C. ROLFS.) Bol. de Agr. Zootech. e Vet. **5**(10). 1932.
The citrus export problem. (With C. ROLFS.) Brasilian Bus. **12**(11). 1932.
Educação Agricola e Progresso Economico. (With C. ROLFS.) O Campo **3**(1): 90–91. (2): 72–74. 1932.
O Abacate; Variedades com pedigree, Sua Cultura. (With C. ROLFS.) Estado de Minas Geraes. Sec. da Agr. Serie Agr. No. 22. 23 pp. 1934.
Domesticating anti-leprie species in Brasil. (With C. ROLFS.) Leprosy Review **9**(3/4). 1938.

STUDIES IN THE FUSARIUM DAMPING-OFF OF CONIFERS. III. RELATION OF TEMPERATURE AND SUNLIGHT TO THE PATHOGENICITY OF FUSARIUM[1]

HOWARD TINT[2]

(Accepted for publication January 15, 1945)

Comparatively little attention has been given to the relation of temperature and sunlight to damping-off as predetermining conditions affecting the levels of pathogenicity in the host-pathogen relationship. The close interrelationship in the field between temperature, sunlight, and moisture factors has made it difficult to interpret observations in terms of any one factor. Only when these conditions are controlled and varied independently in the laboratory, can their relations to the disease be interpreted.

GENERAL MATERIALS AND METHODS

The selection of hosts and damping-off fungi was based upon results from previous experiments (22, 23). In the reports on these tests, sources of seeds and of the Fusarium lines were described in detail, as well as standard methods of seed sterilization, selection of monosporous cultures of virulent strains of fungi, and preparation of inoculum and glycerophosphate nutrient solutions for the hosts and fungi. Damping-off losses were considered in two categories, emergence loss and post-emergence damping-off. Where experimental procedure required occasional departures from the standard methods of these early experiments, the alternative treatments are described.

All seedlings were grown in a low-humidity greenhouse, thermostatically adjusted to maintain a daily mean temperature range of 68–75° F., in those seasons of the year when such control was possible.

Determinations of pH were made potentiometrically with quinhydrone and saturated calomel half-cells, and all solutions were adjusted in acidity with 0.1 N HCl and 0.1 N NaOH.

TEMPERATURE

Various writers have considered effects of soil temperature on seed germination and early growth of forest-tree seedlings (1, 6, 14). Excessively high temperatures following high insolation exert lethal effects upon coniferous seedlings (2, 9, 25). Less attention has been given to the effects of moderate temperatures in predisposing seedlings to damping-off, although it has been generally recognized that temperature variations influence the activities of damping-off and other soil organisms (17, 21).

Fusarium species in general are adapted to a wide range of temperature, but usually develop best at relatively high temperatures, with optima in the

[1] A portion of a thesis submitted to the faculty of the Graduate School of the University of Pennsylvania, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The greater part of this investigation was carried on during the tenure of a George Leib Harrison Fellowship at the University of Pennsylvania.

range 20–35° C. (12, 16, 26). Wollenweber (27) made the generalization that root-invading Fusaria are warm-soil organisms. Other investigators (11) saw a correlation between high-temperature Fusarium wilts and the vegetative development of the parasites, and attributed the influence of temperature to its direct effect upon the fungus. Gifford (4) and Hartley (10) observed parallelism between high temperatures and damping-off of conifers, and Roth (18) demonstrated that damping-off fungi function actively between 11° and 30° C. His two species of *Fusarium* caused appreciable damage to *Picea excelsa* under the protracted influence of temperatures between 24° and 33° C.

The influence of temperature on the development of the disease, and upon the host and pathogen separately, was studied in the greenhouse in temperature chambers described by the writer (24). Air temperatures fluctuated about means at five different levels in a manner resembling diurnal fluctuation in nature. The actual mean temperatures and their standard deviations were as follows: Chamber I, 31.5 ± 1.3° C.; II, 24.6 ± 1.6° C.; III, 20.9 ± 1.4° C.; IV, 16.4 ± 1.5° C.; and V, 10.4 ± 1.6° C. The standard error of any mean was 0.3° C. These values were independent of the greenhouse temperatures.

The first inoculation tests (Series 1) were upon *Pinus resinosa* in quartz-sand cultures. Two four-inch pots, inoculated with cultures of *Fusarium oxysporum*, and two control pots, treated with sterile rice-mush, were in each chamber. Infested pots were incubated for 5 days at room temperature, after which 50 surface-sterilized seeds of the host were sown in each and covered with sterile sand. The pots were then placed in the apparatus and kept uniformly moist by frequent watering with the standard nutrient solution adjusted to pH 6. Data recorded for inoculated and control pots are in tables 1 and 2.

In table 1 are listed the cumulative percentages of seedlings emerging after various periods, up to 125 days, after placement of pots in the chambers. Since emergence had not been completed by 125 days in Chambers IV and V, it was necessary to determine the number of viable seeds remaining in these pots, in order to distinguish between lack of emergence due to inoculation and that due to physiological effects upon the seeds of the temperature alone. The sand in all the pots was sifted through a screen and all unopened seeds recovered were again surface-sterilized and planted in sterile agar in Petri dishes. The plates were incubated at room temperature for 15 days, and the additional germination was used to correct the percentage emergence of the temperature treatments. The corrected emergence percentages are in table 2, which includes the percentage reduction in emergence due to the inoculation, based upon the corresponding controls as standards, and also the total post-emergence losses.

The optimum temperature range for the emergence of the host was 16–20° C., higher values reducing germination, while lower temperatures slowed down its rate. Reduction in emergence due to the effects of the inoculations

TABLE 1.—*Emergence percentages of seedlings of Pinus resinosa grown in control and inoculated quartz-sand cultures at various temperatures. Data are averages of duplicate trials*

Culture	Temperature, degree C.	Emergence percentages from 9 to 125 days after planting of seeds									Total emergence (per cent)
		9	12	14	16	18	40	75	100	125	
Control	31.5	6	13	17	21	24	.....	.....	.....	.....	24
	24.6	5	27	32	35	.....	.....	.....	.....	.....	35
	20.9	1	39	57	65	73	75	75	76	.....	76
	16.4	.....	.....	.....	2	5	24	44	56	70	70
	10.4	.....	.....	.....	.....	.....	3	7	9	12	12
F. oxysporum	31.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	0
	24.6	4	9	11	.....	.....	.....	.....	.....	.....	11
	20.9	4	13	16	24	27	29	31	.....	.....	31
	16.4	.....	.....	.....	4	6	27	38	47	52	52
	10.4	.....	.....	.....	.....	.....	3	7	12	14	14

was directly correlated with temperature, maximum losses occurring in the warmest chamber and minimum in the coldest. A similar correlation with temperature was evident for post-emergence losses.

Some seedlings failed to survive in the control pots, particularly at the warmer levels. *Fusarium oxysporum* and other fungi and bacteria were isolated from such dead seedlings. Nutrient-saturated cultures maintained for a long time in closed chambers at high temperatures provided a highly suitable environment for the development of a fungal flora. Whether failures to survive in such controls were due to lethal effects of temperature upon the host, to the saprophytic organisms entering upon death of the host, or to direct pathogenic attack by these organisms upon the weakened host, could not be determined. Infection of controls with *F. oxysporum* could be directly attributed to contamination as a result of the surface-watering of inoculated and control pots in close proximity. By exercising special care in handling and watering pots while in the chambers, losses in control pots in later tests were reduced to comparatively low levels.

TABLE 2.—*Corrected emergence percentages and post-emergence losses in seedlings of Pinus resinosa grown in temperature chambers*

Culture	Temperature, degrees C.	Corrected percentage emergence	Emergence as percentage reduction of control	Percentage post-emergence loss
Control	31.5	24	.....	61.1
	24.6	35	.....	27.9
	20.9	76	.....	11.9
	16.4	77	.....	1.3
	10.4	74	.....	0.0
F. oxysporum	31.5	0	100.0	.....
	24.6	11	68.6	100.0
	20.9	31	59.2	89.6
	16.4	54	29.8	8.7
	10.4	70	5.4	0.0

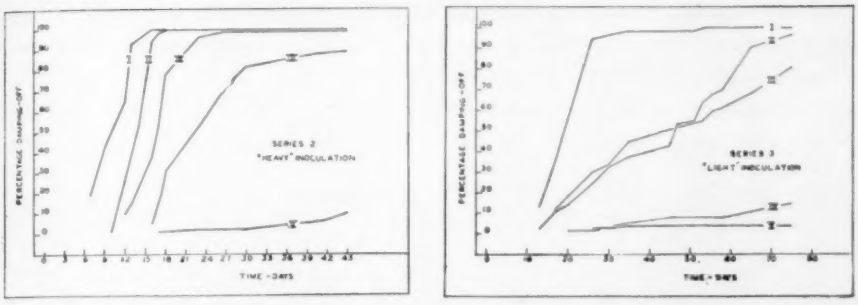


FIG. 1. Effects of temperature on damping-off in seedlings of *Pinus resinosa* in quartz-sand cultures with "heavy" inoculations and with "light" inoculations of *Fusarium oxysporum*.

Since seeds germinated at different rates in the various chambers, the emerged seedlings were exposed for different lengths of time to the fungus. Therefore, the degrees of post-emergence attack at the several temperature levels were not wholly comparable. In order to allow uniform exposure of the seedlings with respect to time, two additional experiments were made (Series 2 and 3). Seeds of *Pinus resinosa* were germinated under sterile conditions and the seedlings transplanted, when their radicles were 1–3 cm. long, to inoculated and control pots which were then placed in the chambers. In Series 2, the standard moderately heavy inoculation procedure was employed. In Series 3, this method was modified as follows: the contents of a flask of rice inoculum were shaken with 50 cc. of distilled water, and the suspension was then used to inoculate two pots of sand. Controls were similarly prepared with a suspension from sterile steamed rice. Thus, inoculation was relatively light in Series 3. Damping-off losses were recorded periodically during 45 days in Series 2 and for 75 days in Series 3. The results are in figure 1.

Upon completion of these tests, the seedlings were removed from the control pots and certain growth responses to the different temperatures were

TABLE 3.—*Effects of temperature upon the growth of seedlings of Pinus resinosa in quartz-sand cultures*

Series and duration	Temperature, degrees C.	Number of seedlings measured	Average length in millimeters		Average weight in milligrams		Ratio green to dry weight	Survival in percentage of original stand
			Roots	Hypocotyls	Green	Dry		
Series 2, 45 days	31.5	19	14.7	40.7	50.5	3.8	14.5	78.0
	24.6	30	15.8	40.9	45.8	5.0	9.2	100.0
	20.9	30	19.7	42.6	43.8	5.3	8.3	98.0
	16.4	30	17.0	39.4	40.7	5.4	7.5	100.0
	10.4	30	16.3	36.9	38.1	4.9	7.8	100.0
Series 3, 75 days	31.5	12	18.0	53.0	57.5	5.0	11.5	56.0
	24.6	20	19.1	47.8	45.9	6.7	6.8	84.0
	20.9	25	19.6	49.6	41.9	8.1	5.2	92.0
	16.4	27	18.3	49.1	41.2	7.3	5.6	100.0
	10.4	30	19.2	44.8	40.2	7.4	5.4	100.0

measured (Table 3). Final stands were expressed as percentages of the original plantings. Representative sets of seedlings from both series were photographed (Fig. 2).

Post-emergence losses in both series were correlated with temperature, as was emergence reduction in Series 1. Most rapid and greatest losses

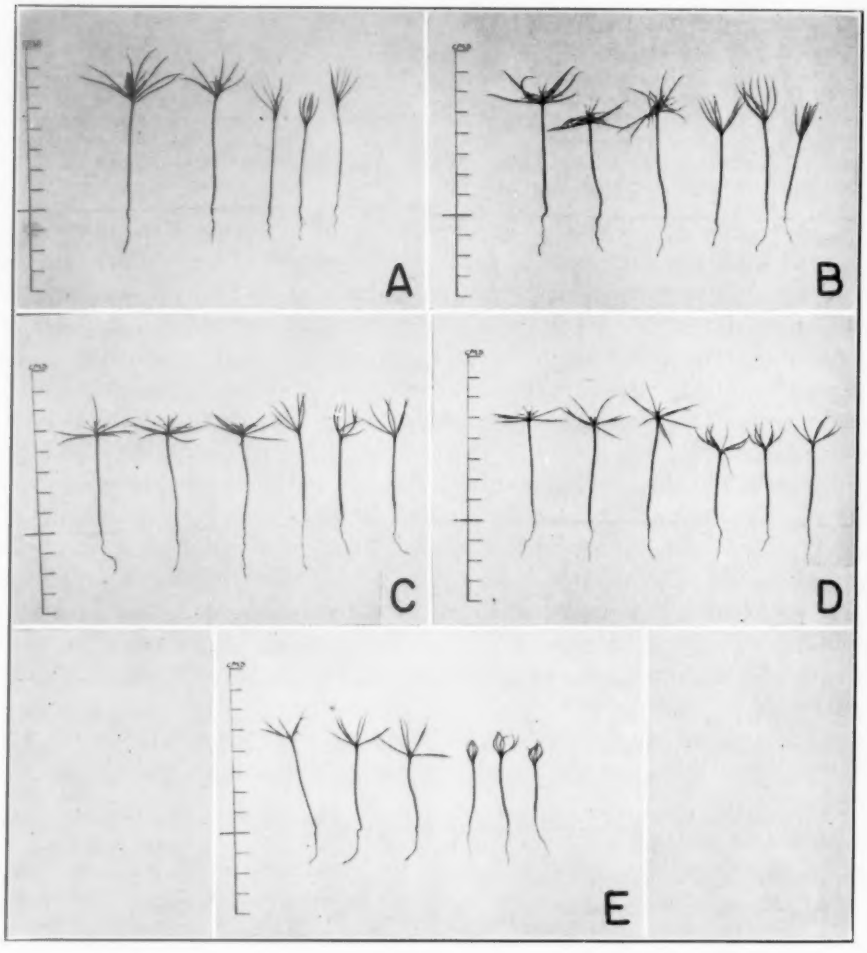


FIG. 2. Representative seedlings of *Pinus resinosa* grown in temperature chambers: A. Chamber I, 31.5° C.; B. Chamber II, 24.6° C.; C. Chamber III, 20.9° C.; D. Chamber IV, 16.4° C.; E. Chamber V, 10.4° C. The 3 larger seedlings on the left in each set are from Series 3 (75 days duration) and those on the right from Series 2 (45 days). Scale in centimeters.

occurred in the warmest chamber; with decreasing temperatures, total losses became less and seedlings were attacked less rapidly. In the same experiment, variations in temperature affected the development of the host. The most rapid growth of tops, as expressed by elongation of hypocotyls and development of leaves, occurred in direct relation to increasing temperatures

(Table 3 and figure 2). The "warm" plants, however, had weaker stems and less pigment than the "cold" ones. The characteristic increasing succulence with rise in temperature was further reflected in the ratios of green to dry weight of the seedlings. The ratios were higher at the higher temperatures.

In both series, final stands of controls were reduced at the higher temperatures. Since some dead seedlings from these controls were infected by contaminating organisms and others were free of infection, it was not possible to ascertain whether the total reduction was due to infection by these contaminants or to lethal effects of the high temperatures. If the former was the case, then unquestionably the higher temperatures predisposed the control seedlings to invasion.

Temperature effects on the growth of *Fusarium oxysporum* were tested in Petri dishes of potato-dextrose agar, adjusted to a final pH of 6 and incu-

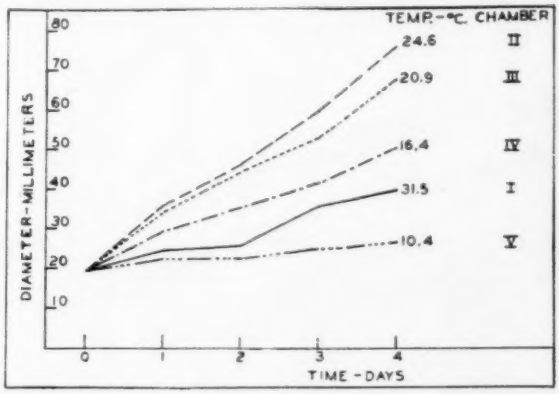


FIG. 3. Effects of several levels of temperature on the growth of *Fusarium oxysporum* on potato-dextrose agar.

bated 24 hours at room temperature before distribution to the chambers. The 5 dishes in each chamber were covered by continuously moistened cloth to equalize as far as possible the humidities at the different temperature levels. All the chambers were given normal exposure to light in order to subject the fungi to the same conditions which would prevail during the inoculation tests, although less fluctuating temperature equilibria could have been obtained in darkness. Colony diameter was measured after each 24-hour period for four days. The daily average diameters at each level of temperature are summarized in figure 3.

The fungus grew over the entire temperature range. Both in daily-increment and total-growth measurements, its maximum growth was at approximately 25° C., growth was slight at 31.5° C. and minimum development was at 10.4° C. The optimum was generally comparable with that of 25°–30° C. reported for the growth in pure culture of *Fusarium oxysporum* isolated from potato-wilt by Goss (5), although he demonstrated that the value varied with the substrate.

SUNLIGHT

The relationship of light to the damping-off problem is an indirect one. Diurnal and seasonal variations in insolation are the main sources of the temperature and moisture fluctuations that occur in the field and affect seedling development (1). Furthermore, light affects growth and survival directly (3, 7, 15, 19, 20). Smith (21) pointed out that the heavy shading of seedlings retards growth and prolongs succulence in a manner that may contribute to damping-off losses. Since nursery practice commonly employs shading in seedling beds, further knowledge is essential on the direct relation of light to damping-off.

The effects of variations in light intensity on the damping-off of coniferous seedlings were studied in the greenhouse. Slotted screens provided varying degrees of illumination (Fig. 4). These were preferred to shading

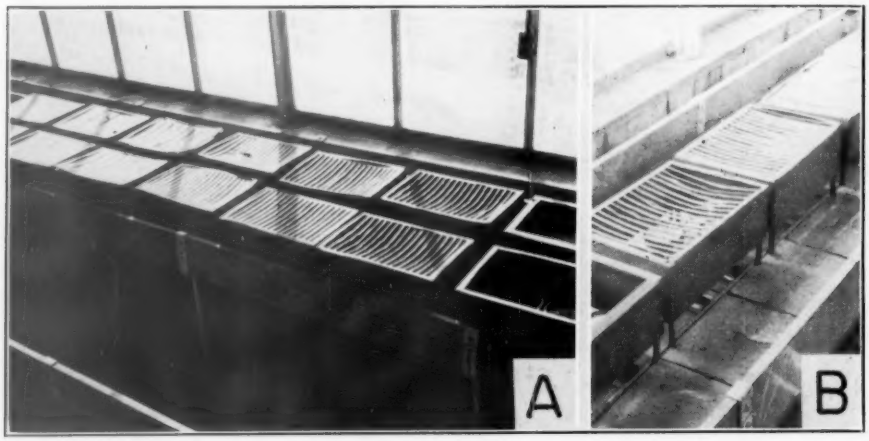


FIG. 4. A. Shading-screens providing varying degrees of radiation-intensity ranging from 0 per cent, upper left, to 100 per cent, lower right. B. View showing manner of supporting frames on legs.

cloths since lath shades are more common in nursery practice. They also provided conditions more closely resembling those under a natural canopy of vegetation, where plants are alternately exposed to high and low light intensities. The panes of the greenhouse were of ordinary clear glass, allowing most of the light to pass through.

The screens and supporting frames ($12 \times 18 \times 6$ inches) were stiff corrugated paper and were painted on all surfaces with nonreflecting black paint. Each frame was supported on legs to raise its lower edge 3 inches above the surface of the bench and permit free circulation of air. At the same time the light-intercepting area was thus sufficiently elevated over the surfaces of the pots so that light intensity, measured with a photo-electric cell (Weston) was uniform at all locations in the seedling level. The width and spacing of the openings of the screens were planned to permit, after calibration with the photometer, various percentages of the light-intensity in the open frame:

75 per cent, 50 per cent, 25 per cent, 12 per cent, 6 per cent and 0 per cent. (See Fig. .4) Each shade frame covered two 7-inch pots.

Duplicate inoculations were made in autoclaved soil (3 parts loam, 2 sand, and 2 leaf-mold) with *Fusarium oxysporum, F. vasinfectum* and *F. avenaceum,* and each pair was tested under a single frame at each level of light-intensity. Fifty surface-sterilized seeds of *Pinus resinosa* and of *Pinus sylvestris* were sown in each inoculated pot after 5-days' incubation of the fungus. Post-emergence losses of seedlings through damping-off were recorded for 30 days. The tests were carried out on the south side of the greenhouse, the apertures of the screens arranged at right angles to the path of

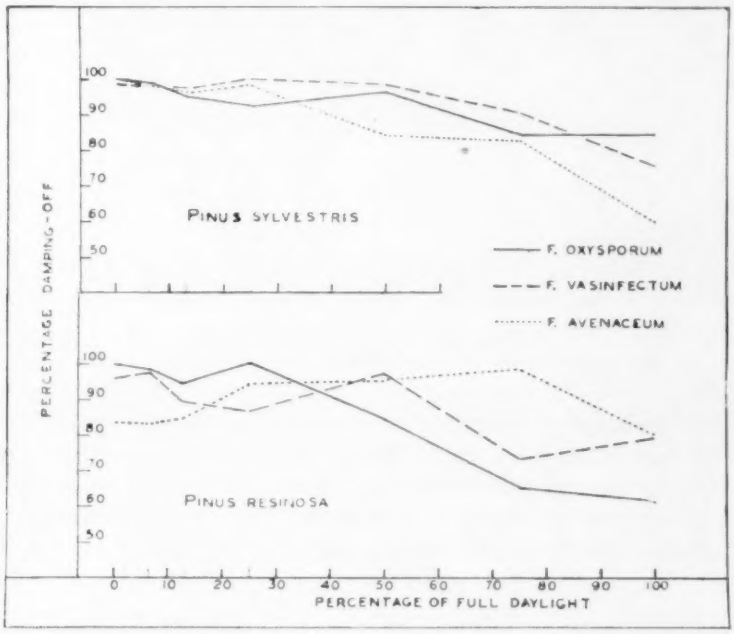


FIG. 5. Relation of light-intensity to damping-off of *Pinus resinosa* and *Pinus sylvestris* in sand cultures inoculated with *Fusarium oxysporum, F. vasinfectum,* and *F. avenaceum.* Data are based upon duplicate trials.

the sun. Damping-off losses in relation to light-intensities are represented in figure 5.

An additional set of uninoculated seedlings was tested, in duplicate pots, in the light-intensity range. The growth characteristics were measured 30 days after emergence (Table 4); and representative seedlings of both hosts grown under each light intensity are shown in figure 6. Succulence in both hosts, measured in terms of hypocotyl elongation and ratio of green to dry weight, increased with decreasing light-intensities. Root length was optimum for *Pinus resinosa* in 50 per cent and 75 per cent light and for *P. sylvestris* in 75 per cent light. The differences in root length, however, were probably an index to temperature variations due to the different degrees of insolation, although the practice of watering frequently during the day,

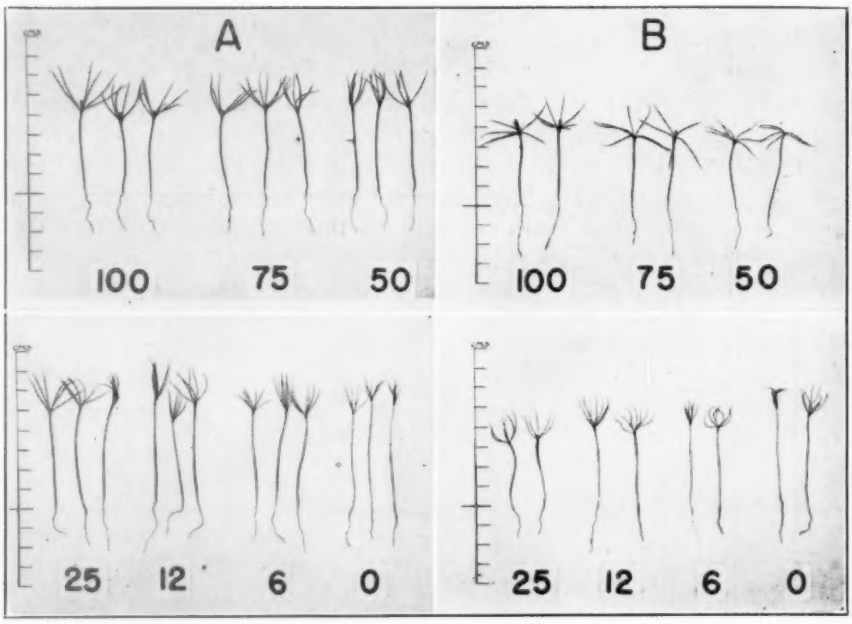


FIG. 6. Representative seedlings of *Pinus resinosa* (A) and *Pinus sylvestris* (B) grown for 30 days under different light-intensities (percentages of full light given numerically).

keeping soil-moisture content uniform in all pots, tended to keep temperatures from fluctuating too widely. Below-surface ($\frac{1}{4}$–$\frac{1}{2}$ inch) temperatures of the soils on the average varied from 18° to 23° C., and the maximum attained in any pot was 28° C. in pots exposed to full maximum solation.

The effects of similar light-intensities upon the growth of the fungi were measured. Lundegårdh (13) cited instances of the penetration of light rays

TABLE 4.—*Relation of light intensity to the growth of Pinus resinosa and Pinus sylvestris in autoclaved-soil cultures. Duration of test 30 days following emergence*

Plant	Percentage of full light	No. of seedlings measured	Average length in millimeters		Average weight in milligrams		Ratio of green to dry weight
			Roots	Hypocotyls	Green	Dry	
Pinus resinosa	0	22	17.1	50.8	30.5	2.4	12.7
	6	30	17.6	51.9	44.4	4.2	10.6
	12	30	16.1	48.1	43.0	4.7	9.2
	25	30	17.1	49.7	43.2	4.4	9.8
	50	30	19.9	46.2	43.4	5.3	8.2
	75	30	19.8	42.7	43.4	5.9	7.4
	100	30	16.9	38.2	44.0	5.8	7.6
Pinus sylvestris	0	24	17.0	45.8	35.0	2.7	13.0
	6	23	15.7	41.2	32.7	2.3	14.2
	12	22	16.0	42.0	32.5	3.0	10.8
	25	24	15.1	37.2	27.5	3.1	8.9
	50	19	13.1	36.2	21.1	3.7	8.4
	75	21	20.2	37.6	37.7	4.6	8.2
	100	22	18.2	35.9	37.5	4.9	7.7

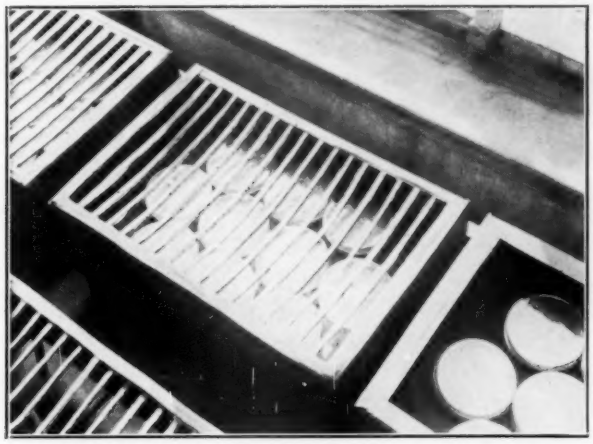


FIG. 7. Adaptation of screens for testing the relation of light intensity to the growth of fungi in Petri dishes.

below the surfaces of soils, measured in terms of the photosynthetic activities of soil algae growing at depths of several centimeters. Therefore, in the present experiments, the effects of light-intensity on the growth of the fungi probably bore some relation to the amount of disease they caused. Petri dishes of potato-dextrose agar were seeded with the fungi, five plates of each organism for each screen, and the plates were placed upon platforms at the same distance below the screens as were the soil-surfaces in the inoculation tests (Fig. 7). After exposure for 4 days, the average diameter of the colonies under the various screens was measured (Fig. 8). Growth of each fungus generally increased with reduction in light-intensity, agreeing with the observations of Harter (8), that the vegetative growth of various Fusaria was more abundant in the dark.

Under the conditions of the experiment, the effects on fungus growth of differences in temperature induced by different light-intensities could not

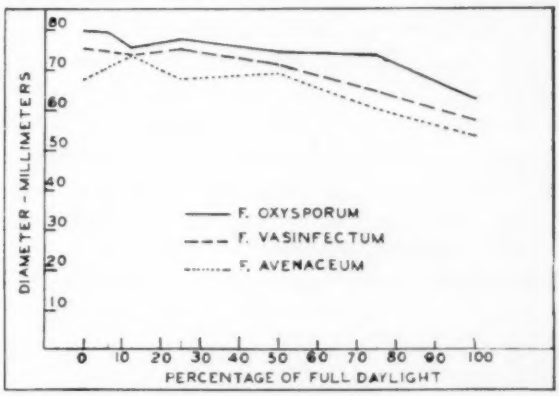


FIG. 8. Effects of light intensity on the growth of three species of *Fusarium* on potato-dextrose agar.

be wholly evaluated. Although greenhouse temperatures were maintained at 20–24° C., undoubtedly increasing insolation elevated temperatures beneath close-fitting Petri-dish covers. However, at no time were lethally high temperatures observed in any plates during the test, and periods of exposure of the plates to direct radiant heat, considered on a 24-hour basis, were relatively short.

DISCUSSION

Damping-off of *Pinus resinosa,* caused by ***Fusarium oxysporum,*** proved to be directly related to temperature. While high temperatures hastened the development of the host, its succulence also increased, so that its resistance to the fungus was lowered. Thus emergence and post-emergence losses were highest at temperatures beyond the optimum for fungus development. However, on the whole, the variation in losses in relation to temperature appeared to depend upon its effect simultaneously upon both host and pathogen. Increase in losses with mounting temperatures showed the same general progression with time in experiments differing in amount of inoculum, thus indicating a host response. On the other hand, the differences in the relative positions of the curves of losses between "heavy" and "light" inoculations, suggested a relation between the virulence of the fungus and the particular temperature conditions.

An important fact demonstrated by these experiments is the apparent existence of a critical temperature below which the host is able to grow and escape infection in infected soil. Roth (18) also described damage caused by Fusarium species only at high temperatures at which *Pythium* and *Rhizoctonia* were relatively harmless. In the present experiments, in both light and heavy inoculations of *Pinus resinosa* seedlings grown at an average temperature of approximately 10° C., losses were negligible. This fact showed a striking parallelism with other Fusarium wilts whose reactions to soil temperatures have been studied (11). These reports have also established minimum temperatures below which the hosts have grown fairly well and have escaped serious infections: for example, tomato wilt, 19° C.; cabbage yellows, 17° C.; flax wilt, 14° C. This phenomenon obviously is directly related to the thermophilic nature of the Fusaria generally, as has already been mentioned. The lowest temperature established in the apparatus of the present experiments, 10° C., probably did not represent the actual critical point. At an average of 16° C., the next highest temperature available, losses were low during the 75 days following a light inoculation of the fungus, and relatively high 45 days after a heavy inoculation. The critical point was probably between 10° and 16° C.

The influence of light upon damping-off was related to its effect upon the host and the pathogen. With increasing shade, both the succulence of the host and the growth of the fungi became greater, and both apparently contributed to increasing damping-off losses. These results could be distinguished from any possibly caused by secondary effects of variations in the insolation. Two factors which vary primarily in the field with the amount

of sunlight are temperature and moisture. In these experiments, moisture variation was minimized through the practice of frequent and uniform watering. The abundant moisture further served to check rising temperatures with increasing insolation. Since maximum temperature variations were recorded for non-shaded pots, if the light experiments were being mainly influenced by secondary temperature variations, then the greatest losses could be expected in the unshaded pots. However, the results indicated that the converse was true, best stands being obtained in full light. This indicated that light conditions were affecting the results independently of secondary temperature effects. In the field, however, unquestionably beneficial effects of full light are lost. Moisture control becomes more difficult with higher insolation, and the higher rate of evaporation which results causes the soil to dry out more rapidly. This, in turn, permits higher soil temperatures and increases possibilities of fungus attack.

SUMMARY

The relation of temperature variations to damping-off and to the growth of the host and pathogen was tested in control equipment.

Optimum growth of *Fusarium oxysporum* was favored by a temperature of approximately 25° C. Emergence of *Pinus resinosa* was reduced lethally by some higher temperatures in the range tested, whereas its rate was slowed down by the lower levels. In post-emergent development, rapidity of growth and succulence of *Pinus resinosa* increased with rising temperatures.

Increase in emergence losses and post-emergence damping-off of *Pinus resinosa* was directly correlated with increasing temperatures. Both types of losses were relatively negligible at an average temperature of 10° C., regardless of the amount of the inoculum used. This suggests the existence of a critical temperature below which *Pinus resinosa* may grow and escape infection by *Fusarium oxysporum,* an organism which otherwise causes relatively high losses.

Damping-off losses of *Pinus resinosa* and *Pinus sylvestris* were in direct relation to decreasing light-intensities, maximum final stands being obtained in full light. This condition was correlated with the increase in succulence of the hosts and in growth of three Fusarium lines used in the inoculations, with decreasing light-intensities.

BOTANICAL LABORATORY,
UNIVERSITY OF PENNSYLVANIA,
PHILADELPHIA, PA.

LITERATURE CITED

1. ADAMS, W. R. Studies in tolerance of New England forest trees. XI. The influence of soil temperature on the germination and development of white pine seedlings. Vt. Agr. Exp. Stat. Bull. 379. 1934.
2. BAKER, F. S. Effects of excessive high temperatures on coniferous reproduction. Jour. For. 27: 949–975. 1929.
3. GAST, P. R. Studies on the development of conifers in raw humus. III. The growth of Scots pine (*Pinus silvestris* L.) seedlings in pot cultures of different soils under varied radiation intensities. Meddelanden från Statens Skogsforsoksanstalt 29: 587–682. 1937.

4. GIFFORD, C. M. The damping-off of coniferous seedlings. Vt. Agr. Exp. Stat. Bull. 157: 143–171. 1911.
5. GOSS, R. W. Fusarium wilts of potato, their differentiation and the effect of environment upon their occurrence. Amer. Potato Jour. **13**: 171–180. 1936.
6. HAASIS, F. W. Germinative energy of lots of coniferous-tree seed as related to incubation temperatures and to duration of incubation. Plant Physiol. **3**: 365–412. 1928.
7. HAIG, I. T. Factors controlling initial establishment of western white pine and associated species. Yale Univ. School of For. Bull. 41. 1936.
8. HARTER, L. L. Influence of light on the length of conidia in certain species of Fusarium. Amer. Jour. Bot. **26**: 234–243. 1939.
9. HARTLEY, C. Stem lesions caused by excessive heat. Jour. Agr. Res. [U.S.] **14**: 595–604. 1918.
10. ———. Damping-off in forest-nurseries. U. S. Dept. Agr. Bull. 934. 1921.
11. JONES, L. R., J. JOHNSON, and J. G. DICKSON. Wisconsin studies upon the relation of soil temperature to plant disease. Wis. Agr. Exp. Stat. Res. Bull. 71. 1926.
12. LINDFORS, T. Einige Kulturversuche mit Fusarium-arten in Nahrlosungen von verschiedener H-ionen Konzentrationen. Botaniska Notiser **2**: 161–171. 1924.
13. LUNDEGÅRDH, H. Die Bedeutung des Kohlensauregehalts und der Wasserstoffionkonzentration des Bodens für die Entstehung der Fusariosen. Bot. Notiser **1**: 25–52. 1923.
14. MCDOUGAL, W. B. The growth of forest tree roots. Amer. Jour. Bot. **3**: 384–392. 1916.
15. MITCHELL, H. L. Pot culture tests of forest-soil problems with observations on the effect of varied solar radiation and nutrient supply on the growth and nitrogen content of scots and white pine seedlings. Black Rock Forest Papers Bull. 5. 1934.
16. MOORE, E. S. The physiology of *Fusarium coeruleum*. Ann. Bot. **38**: 139–161. 1924.
17. PEARSON, C. A. Light and moisture in forestry. Ecol. **2**: 145–160. 1930.
18. ROTH, C. Untersuchungen uber den Wurzelbrand der Fichte. Phytopath. Zeit. **8**: 1–110. 1935.
19. SHIRLEY, H. L. The influence of light intensity and light quality upon the growth of plants. Amer. Jour. Bot. **16**: 354–390. 1929.
20. ———. Light intensity and plant growth in a pine forest. Jour. Agr. Res. [U.S.] **44**: 227–244. 1932.
21. SMITH, L. F. Factors controlling the early development and survival of eastern white pine (*Pinus strobus* L.) in central New England. Ecol. Monographs **10**: 373–420. 1940.
22. TINT, H. Studies in the Fusarium damping-off of conifers. I. The comparative virulence of certain Fusaria. Phytopath. **35**: 421–439. 1945.
23. ———. Studies in the Fusarium damping-off of conifers. II. Relation of age of host, pH and some nutritional factors to the pathogenicity of Fusarium. Phytopath. **35**: 440–457. 1945.
24. ———. An apparatus for the growth of plants under controlled temperatures. Phytopath. **35**: 511–516. 1945.
25. TOUMEY, J. W., and E. J. NEETHLING. Some effects of cover over coniferous seed beds in southern New England. Yale School For. Bull. 9. 1923.
26. TU, C. Physiologic specialization in Fusarium species causing headblight of small grains. Minn. Agr. Exp. Stat. Tech. Bull. 74. 1930.
27. WOLLENWEBER, H. W. Pilzparasitare Welkekrankheiten der Kulturpflanzen. Ber. Deut. Bot. Gesell. **31**: 17–34. 1913.

AN APPARATUS FOR THE GROWTH OF PLANTS UNDER CONTROLLED TEMPERATURE LEVELS

HOWARD TINT

(Accepted for publication January 15, 1945)

INTRODUCTION

Many descriptions have been published of control chambers suitable for experiments on the relation of temperature to plant growth.[1, 2, 3] For the most part, however, the construction and maintenance costs of apparatus limit its general use to relatively few institutions. While such equipment has permitted plant studies under a wide range of conditions controlled by dependable instruments, some problems may be explored in equipment just as adequate for the particular purpose for which it is designed, at relatively much lower expense.

Most apparatus permitting temperature control has been constructed according to the incubator principle, providing single temperatures which are constantly maintained. However, this is in effect not comparable with natural conditions, where temperatures change according to seasonal conditions and fluctuate daily within seasonal levels through the influence of solar radiation. In order to study the temperature relations of plant disease, for example, and to plan experiments comparable to field conditions and upon which predictions and recommendations for nursery practice may be based, temperatures controlled at various levels should be permitted to fluctuate in a similar manner.

In the course of an investigation of the temperature relations of the Fusarium damping-off disease of coniferous seedlings, the writer[4] constructed and used an apparatus which successfully met these requirements and also embodied certain elements of design which made it adaptable to diverse problems in plant physiology, plant pathology, agronomy, etc.

DESCRIPTION OF APPARATUS

The apparatus, in its basic form, is a rectangular, insulated chamber, divided into compartments and bearing a heating unit at one end and a refrigeration unit at the other. Air circulates freely among the compartments, and in operation an air-temperature gradient is thus established over the length of the chamber, ranging from warmest in the compartment adjacent to the heater to coldest at the refrigerator end. Upon exposure of the interiors of the compartments to normal daylight, through glass tops, the

[1] Crocker, W. Organization, equipment, dedication. Contr. Boyce Thompson Inst. **1**: 9–52. 1925.

[2] Davis, H. R., and D. R. Hoagland. An apparatus for the growth of plants in a controlled environment. Plant Physiol. **3**: 277–292. 1928.

[3] Johnson, J. Constant temperature and humidity chambers. Phytopath. **18**: 227–238. 1928.

[4] Tint, H. Studies in Fusarium damping-off of conifers. III. Relation of temperature and sunlight to the pathogenicity of Fusarium. Phytopath. **35**: 498–510. 1945

temperature in each chamber then fluctuates about a different level, the greatest deviation from each mean temperature taking place during periods of maximum insolation and the least during periods of darkness.

Details of the construction are in figure 1. The chamber is built of three-fourths-inch white pine lumber, the bottom and four sides being double-walled, with a dead air-space packed with sawdust between. Half-inch plywood panels, inserted into grooves on the inner sides of the chamber, divide it into five compartments, which are covered by panes of glass with adjoining edges made smooth for close-fitting. The edges of the apparatus upon which the covers rest are further insulated with strips of felt. Additional compartments at both ends contain the heating and cooling units. These

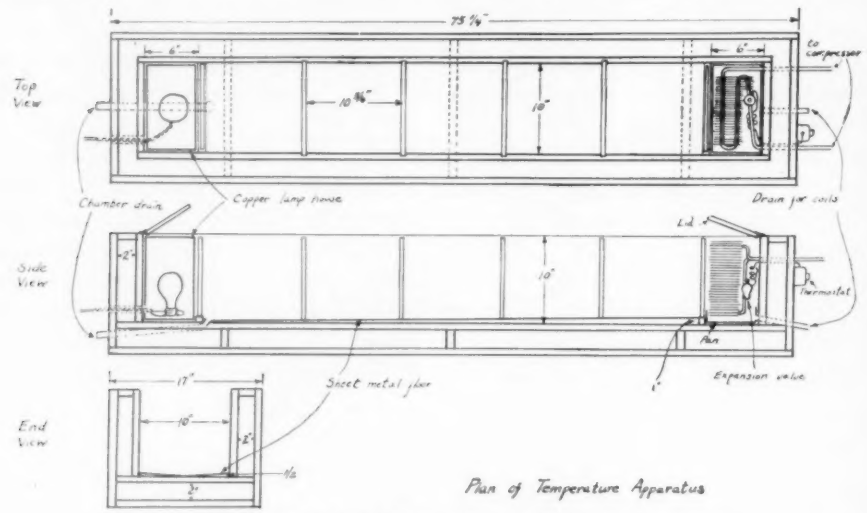


FIG. 1. Working drawings of elevations of the control apparatus.

spaces are separated from the interior of the chamber by additional panels and are covered by wooden doors inserted on the inner edges of the apparatus by hinges (Fig. 2, C, E). All panels are shortened at the top by one-quarter inch to permit free passage of air between adjacent compartments. In addition, the panels separating the spaces containing the heater and refrigerator units are also similarly shortened on the sides. These baffles thus permit free conduction of cold or warm air into the apparatus, at the same time preventing excessive radiation of heat into the chamber adjacent to the heater and freezing of solutions subsequently placed in close proximity to the refrigerator coils. Some panels bear holes permitting additional circulation of air (Fig. 2, B), which tends to space more evenly the temperature increments in the intervening compartments between the two extremes established at the ends.

Galvanized iron on the floor of the apparatus provides a graded surface in two directions (Fig. 1): a one-inch grade over the entire length and a half-inch slope from the edges to the center. Water drains towards an out-

let at one end of the chamber (Fig. 2, C, D). A drip pan and drain are also provided for the compartment containing the refrigeration coils (Fig. 1). Open seams within the chamber are caulked with putty to prevent the entrance of water into the sawdust-filled air space. All interior surfaces are painted with white enamel, for reflection of light within the compartments.

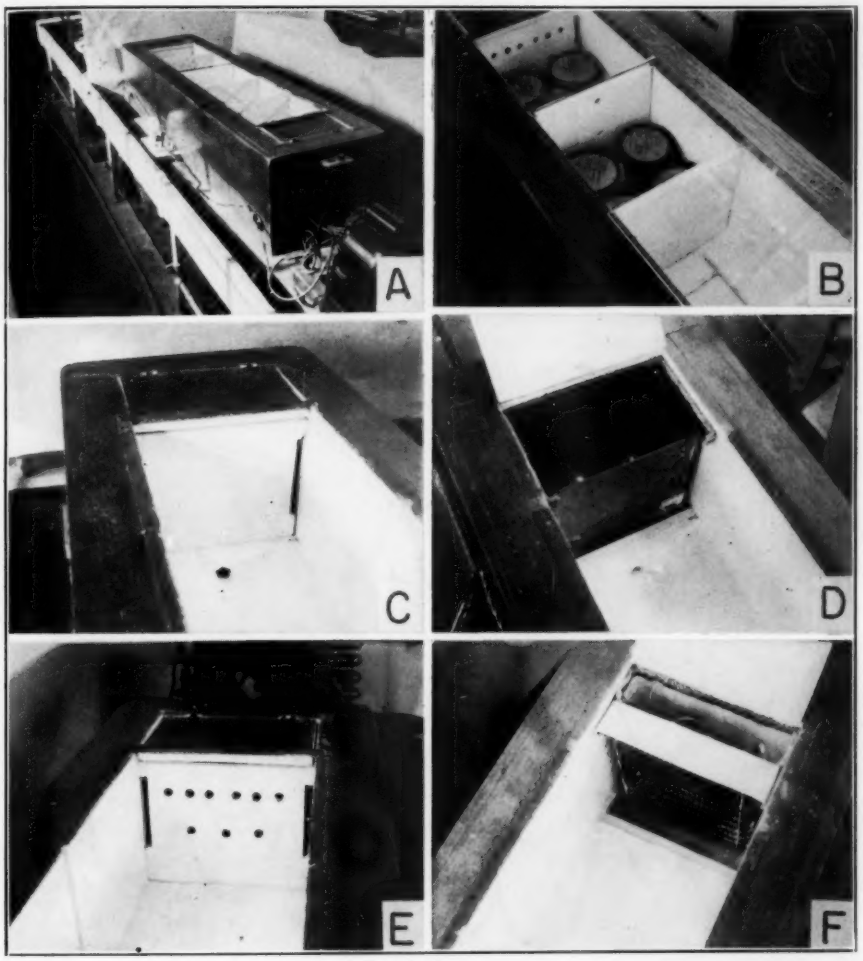


FIG. 2. A. View of entire apparatus. B. Enlarged view of equipment showing manner of disposition of pots of plants in the chambers. The glass cover of the central chamber has been removed. In the lower chamber, the manner of supporting pots on the sloping floor is illustrated. C. Warm end of chamber with lid and baffle in place. D. Same view as C with lid raised and cover of lamp house removed. The aperture for chamber-drain may be seen. E. Cold end of chamber, lid and baffle in place. F. Same view as E with refrigerator coil exposed.

Heat is supplied by electric bulbs housed in a copper container (Fig. 2, D), and the degree is controlled by varying the wattage of the bulbs. For the continuous and uniform supply of heat, the bulbs remain lighted for the duration of an experiment. Since ordinarily they are not visible, it is con-

venient to install in the exterior heater circuit an additional bulb in series (Fig. 2, A). A failure of this bulb to light indicates that the heating bulbs are inoperative.

Cold air is supplied by a small refrigeration machine equipped with thermostatic control, taken from a home-model refrigerator. The normal freezing coil is replaced by a double-pass fin-coil (Fig. 2, F) with an expansion valve control. The coil maintains low temperatures in the adjacent compartment (approximately 8° C. if desired) against the warm air coming from the other end of the apparatus.

The limits of the temperature range that may be established are thus conditioned by the relative degrees of heat and cold which are applied at the ends, and the spacing of the temperatures between these extremes in the intervening compartments is established experimentally by regulating the area through which air may circulate between adjacent chambers.

TEMPERATURE RECORDING

The actual temperatures in each of the chambers, in a 7-day test run with heat from a 20-watt bulb and a specific cold-setting, are shown in figure 3.

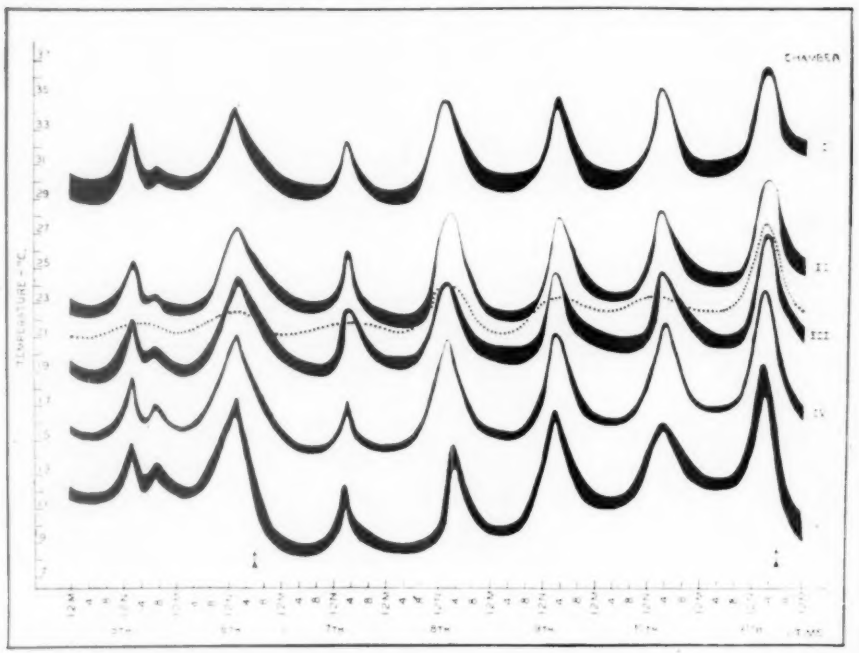


FIG. 3. Temperature record illustrating the levels of temperature secured within the air-control chambers over a period of one week, and the manner in which variation under the influence of insolation took place. Arrows indicate times of defrosting of refrigerator coils. The dotted line records the mean daily temperatures of the greenhouse during this period.

The width of the curve lines indicates the variance between the readings of 2 thermometers, suspended with bulbs at opposite ends of each compartment. Observations were hourly during daylight, and values for hours of

darkness usually were interpolated. In several 24-hour watches, after temperatures reached equilibria in darkness, they remained practically constant until daylight.

Diurnal fluctuations of temperature took place at different levels in the various chambers. There was a slight lack of uniformity for the two coldest chambers, whose values became generally higher as ice was deposited upon the freezing coil. Little change, however, was evident for 48 hours following defrosting (indicated in Fig. 3 by arrows). Consequently, in practice, the coils were defrosted every other day.

The sensitivity to variations in light intensity is evident. Maximum rise in temperature in the various chambers occurred during greatest insolation, bright days showing higher peaks than cloudy days. The sensitivity to light is evident in the record for the 5th day (Fig. 3). The morning and early afternoon were cloudy and dull, causing relatively slight rises in the temperature levels. In the late afternoon, however, the sun appeared very brightly, and as a consequence a secondary temperature rise in all the chambers was evident.

The levels of temperature attained in the several chambers of the apparatus and their fluctuations are independent of the external greenhouse temperature, which was controlled during the test.

The mean temperatures maintained in each chamber for the two days following defrosting—6th day, 6 P.M. to 8th day, 6 P.M.—and their standard deviations were: I, 31.5° ± 1.3° C.; II, 24.6° ± 1.6° C.; III, 20.9° ± 1.4° C.; IV, 16.4° ± 1.5° C.; and V, 10.4° ± 1.6° C. The standard error of any mean was 0.3° C.

COST AND ADAPTABILITY

The cost of the apparatus was comparatively low. Materials used in construction totaled approximately $25.00, and the price of the fin-coil, expansion valve, thermostat, and other installation expenses totaled less than $45.00. The compressor is relatively inexpensive, or else refrigeration may be furnished by other machines which often are standard equipment for many laboratories. Daily maintenance costs are almost negligible.

The equipment, as described, has been adequate for testing the relation of temperature to plant growth and disease,[5] when the particular purpose was to maintain a temperature range, each level of which duplicated natural conditions by increasing within its range under insolation. For other purposes, extensive modifications are possible. Constant temperatures may be secured, either by the exclusion of light, or, if light is essential, by the construction of a cover containing sources of light which produce no appreciable heat, as for instance, fluorescent filament-bulbs. In addition, a rheostat and thermostat included in the heating circuit would provide a more easily controllable source of heat.

[5] See footnote 4.

SUMMARY

An apparatus is described which permits the investigation of plant development simultaneously at several levels of temperature. The design of the equipment allows temperatures in separate compartments to fluctuate within their respective levels in a manner resembling the normal diurnal range of temperature in the field under the influence of varying degrees of insolation.

Details for the construction of the equipment are given. The cost of the materials is relatively low and subsequent maintenance expenditures are negligible.

BOTANICAL LABORATORY,
UNIVERSITY OF PENNSYLVANIA,
PHILADELPHIA, PA.

A PRELIMINARY REPORT ON FURTHER STUDIES OF PHYSIOLOGIC SPECIALIZATION IN USTILAGO HORDEI[1]

T. F. Yu and C. T. Fang[2]

(Accepted for publication January 15, 1945)

INTRODUCTION

In a previous paper (3) was reported an investigation at Nanking University, which led to the discovery of the existence in China of 5 distinct physiologic races of covered smut (*Ustilago hordei* (Pers.) K. and S.) of barley. These races, numbered C–1 to C–5 inclusive, were differentiated on three varieties of barley. On account of the war which forced the removal of Nanking University to Szechwan, the smut material was unfortunately lost during transit. The study was resumed in Kunming, Yunnan, in 1939. The results of the investigations from 1939 to 1943 are presented in this report. The literature on physiologic specialization of *Ustilago hordei* has been reviewed by Tapke (2) and Yu (3), and will not be reviewed again in this paper.

MATERIALS AND METHODS

Numerous collections of *Ustilago hordei* were obtained mostly from southwestern China. Fourteen varieties of barley were inoculated with most of these collections, and 4 barley varieties were selected as differential hosts. Nanking Nos. 368 and 373, which had been used in the previous study (3), were still retained as differential hosts. The two other varieties, Himalaya (C.I. 1312), and Excelsior (C.I. 1248), were received through the kindness of Dr. V. F. Tapke, United States Department of Agriculture. Finland, one of the differential hosts used in the previous investigation, was omitted because of its high degree of sterility when grown in this part of China.

The methods used in inoculation were those described by Tapke (1) in 1935. Inoculated seed was planted at about 2 grams in each of three 3-foot rows, with one or two replications. The smut percentages obtained were based on counts of the total number of heads per plot of 3 rows. Three infection classes were established: resistant class (R), 0–5 per cent smutted heads; intermediate class (I), 5.1–20 per cent smutted heads; and susceptible class (S), 20.1–100 per cent smutted heads.

[1] Paper No. 23 from the Division of Plant Pathology, The Institute of Agricultural Research, National Tsing Hua University.

PHYTOPATHOLOGY extends the courtesy of its journal pages to scientists in other countries who are persevering in research under difficult wartime conditions and are temporarily deprived of the opportunity for membership in the American Phytopathological Society.

[2] The writers take pleasure in acknowledging their indebtedness to Prof. F. L. Tai for the stimulating encouragement during the progress of the investigation; to Messrs. H. R. Wang and S. Y. Yin for assistance in field experiments; and to Drs. L. Ling, Szechwan, and C. S. Wang, Honan, for smut material.

TABLE 1.—*Percentages of smutted heads obtained in 5 varieties of barley inoculated with 9 physiologic races of Ustilago hordei at Kunming, Yunnan, China, 1941–1943*

Name of differential host	Year tested	Physiologic race number of *Ustilago hordei*								
		1	2	3	4	5	6	7	8	9
		Pct.[a]	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Himalaya (C.I. 1312)	1941	0.0	0.0	11.7	0.0	0.0	5.2	.....	0.0	.....
	1942	1.0	0.0	13.8	0.0	0.0	6.4	7.1	4.6	7.1
	1943	0.0	0.0	7.8	0.0	0.0	6.4	7.1	13.8	4.8
	Av.	0.3	0.0	11.1	0.0	0.0	6.0	7.1	9.2	6.0
Nanking No. 368	1941	2.1	0.0	2.3	4.0	0.0	0.0	0.0	9.7	7.5
	1942	0.0	0.0	0.6	4.0	0.0	3.6	0.0	6.7	4.8
	1943	2.0	0.0	1.8	8.8	0.0	3.0	0.9	10.5	5.1
	Av.	1.4	0.0	1.6	5.6	0.0	2.2	0.3	9.0	5.8
Nanking No. 373	1941	0.0	0.0	6.3	0.0	11.0	18.6	9.5	8.7	18.6
	1942	0.0	0.0	0.0	6.3	16.6	13.6	5.8	10.1	27.9
	1943	0.0	0.0	0.0	0.0	14.8	8.2	12.3	11.3	20.2
	Av.	0.0	0.0	2.1	2.1	14.1	13.5	9.2	10.0	22.2
Excelsior (C.I. 1248)	1941	5.4	13.7	0.0	0.0	0.0	.....	24.5	3.6	10.0
	1942	6.0	10.7	0.0	0.0	1.5	11.8	21.8	5.1	10.4
	1943	3.6	8.5	4.2	0.0	0.0	12.0	26.1	7.0	13.4
	Av.	5.0	11.0	1.4	0.0	0.5	11.9	24.1	5.2	11.3
Local hulled[b]	1941	39.4	54.6	28.0	47.6	27.1	29.6	28.1	42.9	55.0
	1942	35.3	52.7	38.6	43.1	50.2	31.4	40.5	47.3	33.7
	1943	40.6	37.2	29.1	11.1	36.3	40.6	49.9	25.6	43.8
	Av.	38.4	48.2	31.9	33.9	37.9	33.9	39.5	38.6	44.2

[a] Average percentages of smutted heads of 2 or 3 plots.
[b] Susceptible check variety.

RESULTS

The average percentages of smutted heads produced by the races on the differential hosts in 1941, 1942, and 1943 are recorded in table 1 and the reactions that serve to differentiate the races of *Ustilago hordei* are presented in table 2.

TABLE 2.—*Relative susceptibility of 4 differential hosts to 9 physiologic races of Ustilago hordei*

Differential host	Reaction[a] to each physiologic race								
	1	2	3	4	5	6	7	8	9
Himalaya (C.I. 1312)	R	R	I	R	R	I	I	I	I
Nanking No. 368	R	R	R	I	R	R	R	I	I
Nanking No. 373	R	R	R	R	I	I	I	I	S
Excelsior (C.I. 1248)	R	I	R	R	R	I	S	I	I

[a] R = 0–5 per cent; I = 5.1–20 per cent; and S = 20.1–100 per cent infection.

There are at least 9 distinct physiologic races which can be recognized by their effects on 4 varieties of barley (Table 1). The differences in the infection capabilities are shown more clearly in the following analytical key:

Analytical key for identification of 9 physiologic races of Ustilago hordei

	Race No.
Nanking No. 373 resistant	
Nanking No. 368 resistant	
Himalaya resistant	
Excelsior resistant	1
Excelsior intermediate	2
Himalaya intermediate	3
Nanking No. 368 intermediate	4
Nanking No. 373 intermediate	
Nanking No. 368 resistant	
Himalaya resistant	5
Himalaya intermediate	
Excelsior intermediate	6
Excelsior susceptible	7
Nanking No. 368 intermediate	8
Nanking No. 373 susceptible	9

Race 1 is by far the weakest in pathogenicity. In fact, all of the 14 varieties of barley are resistant to it. Races 4, 8, and 9 produced an intermediate reaction on Nanking No. 368, a hull-less barley introduced from Japan. It has been known as highly resistant to covered smut of barley at many places in China.

Of a total of 84 smut collections, many have been tested for only one or two years and thus data do not permit a detailed analysis of the prevalence and distribution of the races in China. Nevertheless races 1 and 2 evidently are widely distributed in China. The former occurred in 33 and the latter in 19 of the 84 collections.

Hanchen and Trebi barleys, which have been included in the 14 hosts, were highly resistant to almost all the smut collections. They had, however, a few smutted heads when inoculated with certain smut collections. When smut spores from either Hanchen or Trebi were used to inoculate these same hosts respectively an increase in smut resulted. This indicates that some of the smut collections were a mixture of two or more races and that there are possibilities of isolating a race or races to which either Hanchen or Trebi, or both, will not be resistant.

SUMMARY

Eighty-four collections of covered smut of barley were obtained from many places in China. Fourteen varieties of barley were inoculated with most of these collections.

Nine physiologic races of *Ustilago hordei* can be recognized by their parasitic behavior on four varieties of barley. They have been designated races 1 to 9.

Races 1 and 2 were collected more often than the others. But detailed analysis of the distribution and prevalence of physiologic races of *Ustilago hordei* in China cannot be made until more records are available.

It is likely that there are other physiologic races which could be recognized if more collections were made and more differential hosts inoculated.

INSTITUTE OF AGRICULTURAL RESEARCH,
TSING HUA UNIVERSITY.

LITERATURE CITED

1. TAPKE, V. F. An effective and easily applied method of inoculating seed barley with covered smut. Phytopath. **25**: 1038–1039. 1935.
2. ———. Physiologic races of *Ustilago hordei*. Jour. Agr. Res. [U.S.] **55**: 683–692. 1937.
3. YU, T. F. Breeding hulled barleys for resistance to covered smut (*Ustilago hordei* (Pers.) K. and S.) in Kiangsu Province. Nanking Jour. **9**: 281–292. 1942.

APPLICATION OF PENICILLIN TO CROWN GALL

J. G. Brown and Alice M. Boyle

(Accepted for publication February 15, 1945)

The action of crude penicillin on crown gall has resulted in the destruction (Fig. 1, B, C) of the treated galls. The results are noteworthy because the cause of crown gall, *Agrobacterium* (*Phytomonas*) *tumefaciens*, is a Gram-negative bacterium and, as such, member of a group regarded (1, 5, 6) as resistant to the action of penicillin. They also are interesting because crown gall, the disease concerned, is commonly regarded as incurable, although a few chemicals have been reported (2, 3, 4) as curative when properly applied.

The crude penicillin was produced in this laboratory from an improved strain of *Penicillium notatum* obtained from the Northern Regional Laboratory, Peoria, Illinois. The fungus was grown in a simple apparatus (Fig. 2) that can be sterilized in the autoclave.

Media used for growing *Penicillium* were modified Czapek-Dox (1) and corn-grain juice. The latter was prepared by soaking and afterwards simmering for a half-hour 50 g. dry weight of corn grains per l. of tap water. The media were steam-sterilized and filtered before use. As a further precaution, crude penicillin from the cultures was always filtered through a Chamberland or a Mandler candle. Thus made, the cost of the crude penicillin is approximately two cents per quart.

Soft galls were developed on plants of *Bryophyllum* as a result of inoculations with a pure culture of *Agrobacterium tumefaciens*. Crude penicillin that assayed 6 Oxford units was injected hypodermically into galls on young plants and into the stems of the host just below the galls in older plants. Growth of galls on young plants was checked, but the galls were not killed by one treatment by injection (Fig. 1, B, c) although the resulting necrosis was extensive in some cases. In larger galls likewise treated growth was checked just above the needle punctures, which accentuated the irregularity of gall surface.

The galls were then wrapped with cotton wool soaked in crude penicillin. That method of application resulted in retarded growth and browning of the small elevations on the surface of the galls; elsewhere there appeared to be lack of penetration of the penicillin into the gall. Numerous punctures of the galls, made by thrusting a sterile needle through the cotton wrapping, were soon followed by the death (Fig. 1, C, e, f) of the galls. The parenchymatous tissues became brown, vascular strands remained colorless, and the internal necrosis was limited to the tissues of the gall. However, some superficial injury resulted in normal parts of the stem that were in contact with the overlapping cotton wrapping (Fig. 1, C, e, f). Controls were unaffected.

Penicillin has been reported (7) as bactericidal for the crown-gall bacterium *in vitro*. Waksman and co-workers say that the bacterium "may

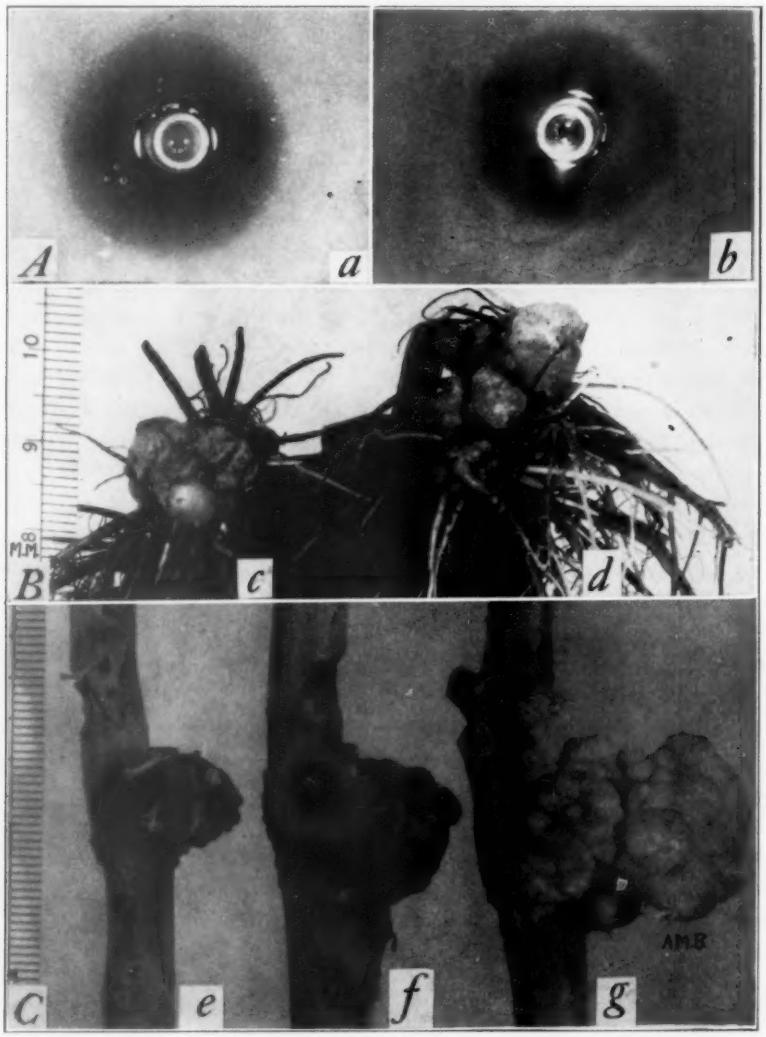


FIG. 1. A. Effect of crude penicillin *in vitro*, a, on *Agrobacterium* (*Phytomonas*) *tumefaciens;* b, on *Staphylococcus aureus;* cultures same age, subjected to penicillin at same time, from same flask, run simultaneously at same temperature. B, C. Effect of crude penicillin *in vivo* on young gall (B) and on older galls (C) of *Bryophyllum;* e, most of gall killed as a result of 4 simultaneous hypodermic injections of penicillin but with diffusion of chemical apparently incomplete; d, control galls that were approximately the same size as galls in e at time of injection; e and f, older galls killed by crude penicillin applied through cotton wrapping; e, longer exposed to air, dry and brown; f, drying and browning; g, control. All three galls same age and approximately same size before treatment.

be considered as fairly resistant when compared with the sensitivity to penicillin of the Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus*

subtilis)." In our tests *in vitro,* limited to the cup-method, little, if any, difference in sensitivity to penicillin was found when *Agrobacterium tumefaciens* was compared with *Staphylococcus aureus.* Perhaps neither the tests reported by Waksman *et al.* nor our tests give a true picture of the comparative sensitivity of the two bacteria. In the former paper it is stated that the test cultures were run at 37° C, the optimum temperature for *Staphylococcus aureus* but the maximum temperature (9° C–12° C above the optimum) for *Agrobacterium tumefaciens,* considerably to the advantage of the former organism. Our cultures were run at room temperature, 22° C–26° C, below the optimum for *Staphylococcus aureus* and partly below the optimum for *Agrobacterium tumefaciens.*

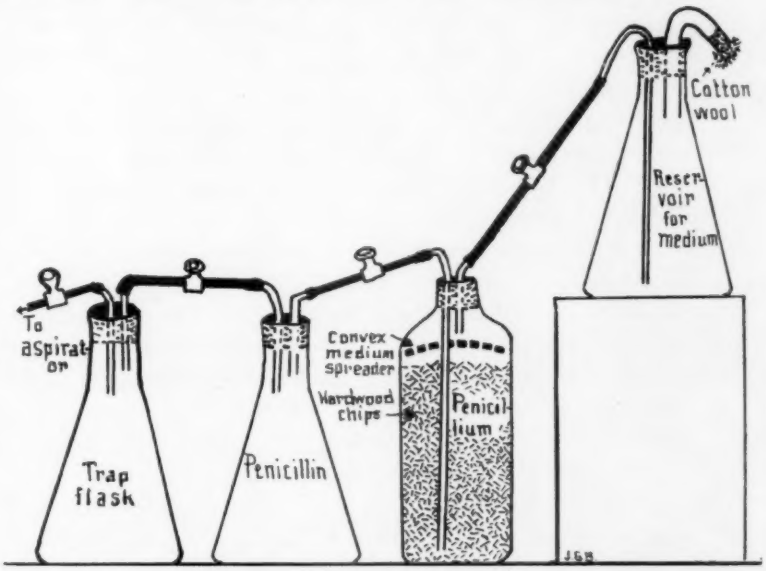


FIG. 2. Diagram of apparatus for producing crude penicillin.

In conclusion it may be remarked that crown gall, practically world-wide in distribution, is particularly serious in the Southwest. The alkaline reaction of the soil, mild climate, and the practice of irrigation favor the development and spread of the bacterium that causes the disease, and the heavy transpiration in a semi-arid atmosphere adversely affects the host. Entire orchards have been destroyed by crown gall. In clean soil the galls usually first appear on the crowns of plants from which the infection later spreads to the roots. The primary source of infection usually can be traced to the nursery. Crude penicillin should prove valuable in combating crown gall of trees and shrubs in which galls are limited to the crown. Possibly it may also be useful in budding and grafting operations in nurseries in which crown gall so frequently has its inception.

UNIVERSITY OF ARIZONA,
TUCSON, ARIZONA.

LITERATURE CITED

1. ABRAHAM, E. P., E. CHAIN, C. M. FLETCHER, A. D. GARDNER, N. G. HEATLEY, M. A. JENNINGS, and H. W. FLOREY. Further observations on penicillin. The Lancet 241(2): 177–189. 1941.
2. ARK, P. A. Chemical eradication of crown gall on almond trees. Phytopath. 31: 956–957. 1941.
3. BROWN, NELLIE A. Colchicine in the prevention, inhibition, and death of plant tumors. Phytopath. 29: 221–231. 1939.
4. ————. The effect of certain chemicals, some of which produce chromosome doubling, on plant tumors. Phytopath. 32: 25–45. 1942.
5. CHAIN, E. Penicillin as a chemotherapeutic agent. The Lancet 240(2): 226. 1940.
6. GARDNER, A. D. Morphological effects of penicillin on bacteria. Nature 146: 837–838. 1940.
7. WAKSMAN, SELMAN A., ELIZABETH BUGIE, and H. CHRISTINE REILLY. Bacteriostatic and bactericidal properties of antibiotic substances, with special reference to plant-pathogenic bacteria. Bul. Tor. Bot. Club 71: 107–121. 1944.

THE MODE OF INFECTION AND THE INCUBATION PERIOD IN THE STEM SMUT OF GRASSES, USTILAGO SPEGAZZINII (U. HYPODYTES)[1]

GEORGE W. FISCHER

(Accepted for publication February 16, 1945)

INTRODUCTION

Stem smut caused by *Ustilago spegazzinii* Hirsch. and *U. spegazzinii* var. *agrestis* G. W. Fisch. and Hirsch. (until recently (9) considered as *U. hypodytes* (Schlecht.) Fr.) is a common disease of many grasses in many parts of the world. Because of its effect on the host plants and the fact that it often attains epiphytotic proportions, the disease has attracted world-wide attention. Many species of grasses, in various genera and tribes have been listed as hosts (3, 5, 6, 8, 11, 13). It has been shown recently, however (9), that some of these records refer to other stem smuts similar in character, but caused by *U. halophila* Speg., *U. nummularia* Speg., and *U. williamsii* (Griff.) Lavrov, species which are morphologically distinct from *U. spegazzinii* and the variety *agrestis*. The morphological modifications of the host caused by stem smut have been especially studied by Feucht (7) and Bond (2), showing that parasitic infection results in the development of sterile culms bearing an excessive number of internodes and leaves in place of an inflorescence. Boss (4) and also Kharbush (10) studied the cytology of the fungus. Berkeley (1) called attention to the perennation of the fungus in the perennial tissues of the host plants, and this has been noted frequently since that time. The general biology of the stem smut fungus on *Elymus arenarius* L. was carefully investigated by Bornhövd (3), including inoculation experiments. She tried seedling infection, blossom infection, and infection of the young shoots, all with negative results. Bond (2) theorized that "infection takes place at the seedling stage rather than later." However, he was unable to germinate the spores of the smut and hence could not prove his theory.

Thus it is seen that in spite of much attention, study, and observation by many persons, the mode of infection in this common stem smut of grasses has thus far remained undiscovered. It is the purpose of this paper to present experimental and observational data which indicate the method of infection and the incubation period of the stem smut of grasses, caused by *Ustilago spegazzinii* and the variety *agrestis*.

OBSERVATIONAL DATA

In 1939 stem smut appeared spontaneously in severe proportions in a large plot of slender wheatgrass, *Agropyron trachycaulum* (Link) Malte,

[1] Grass disease investigations of the United States Department of Agriculture, Agricultural Research Administration, Bureau of Plant Industry, Soils, and Agricultural Engineering, Division of Forage Crops and Diseases, in cooperation with the Soil Conservation Service, Division of Nurseries, and the Washington Agricultural Experiment Station, Pullman, Wash. Published with the approval of the director as Scientific Paper No. 608.

and another of crested wheatgrass, *A. cristatum* (L.) Gaertner, in the Bureau of Plant Industry grass breeding nursery, Pullman, Washington. The plot of slender wheatgrass consisted of 1256 spaced, individual plants, representing the progenies of 248 selfed head selections. The plot of crested wheatgrass consisted of 690 rows, each row resulting from the seed of a single plant selection. Both were seeded in the spring of 1937, and headed out during the summer.

Table 1 shows the incidence of stem smut in the slender wheatgrass, 1937–1940 (after which the plot was destroyed), and table 2 shows the data for the crested wheatgrass, 1937–1943.

No stem smut appeared until the third growing season, in both the slender and crested wheatgrass, and the amount increased sharply in subsequent seasons. These observations are substantiated by similar observations in the Strain Trial Planting of Forage Grasses, in cooperation with the Soil Conservation Service, Pullman, Wash. These grasses were seeded in the

TABLE 1.—*Comparative incidence, on a plant basis, of stem smut (Ustilago spegazzinii var. agrestis) in the same plot of slender wheatgrass during 1937–40*

Year[a]	Plants[b] in plot	Smutted	Smutted
	Number	*Number*	*Per cent*
1937	1256	0	0.00
1938	1256	0	0.00
1939	1241	332	26.75
1940	1234	427	34.60

[a] The plants were transplanted (spaced) to the field in the spring in 1937. They headed out the same season, and, of course, every season since.

[b] The 1256 plants in the plot represented the progenies of 248 selfed head selections, of which 129 selections had more or less smut.

spring of 1939. The first stem smut appeared in 1941, the third season after seeding, with a tremendous increase in 1942, 28 and 70 rows, respectively.

This same observation has been made in the extensive observational row nurseries and the seed increase plots of the Soil Conservation Service, at Pullman, Washington. The first of these nurseries was seeded in the spring of 1936, and a new nursery has been planted each spring since. In these several nurseries and in the seed increase plots, no stem smut (due to *Ustilago spegazzinii* or the variety *agrestis*)[2] appeared before the third growing season.

In addition to the observational data obtained from grass nurseries and seed increase plots, the same minimum three-season interim between time of planting and the first expression of stem smut has been noted in fields of crested wheatgrass in the vicinity of Pullman, Washington. Many fields of this grass have been inspected for stem smut and in every case where it was possible to ascertain the age of the stand, stem smut was found only in fields that were in the third or subsequent heading.

[2] Stem smut due to *Ustilago williamsii* (9) was observed and collected on *Stipa* and *Oryzopsis* in earlier headings, indicating that this stem smut species has a different life history.

TABLE 2.—*Comparative incidence, on a row basis, of stem smut (Ustilago spegazzinii var. agrestis) in the same plot of crested wheatgrass during 1937–43*[a, b]

No. Selection	No. of rows[c]	No. of rows having smut					Rows smutted, 1943
		1939	1940	1941	1942	1943	
							Per cent
50	1	0	0	0	0	0	0
53	3	0	0	0	0	0	0
926	4	0	0	0	2	3	75
1916	11	0	3	7	9	11	100
1917	20	4	17	19	20	20	100
1918	10	3	9	9	10	9	90
1919	12	4	12	12	11	12	100
1920	13	0	8	9	11	11	85
1921	12	0	8	10	12	12	100
1922	15	4	5	5	7	10	67
1923	36	6	18	19	25	27	75
1924	23	11	18	20	21	22	96
1925	19	8	12	13	16	17	89
1926	9	0	4	6	7	8	89
1928	5	0	5	5	5	5	100
1929	7	0	4	4	5	7	100
1931	18	0	4	4	5	8	44
1932	17	3	10	11	13	15	88
1933	4	0	3	3	4	4	100
1934	11	0	5	6	10	11	100
1935	3	0	3	3	3	3	100
1936	7	0	6	6	7	7	100
1937	19	5	17	18	18	18	95
1938	1	0	1	1	1	1	100
1939	17	3	14	16	16	17	100
1940	3	0	3	3	3	3	100
1941	1	0	0	0	0	0	0
1942	6	0	2	4	5	6	100
1944	8	1	1	1	1	2	25
1945	9	0	2	2	3	4	44
1948	7	0	4	6	5	7	100
1950	6	1	4	5	6	6	100
1951	16	2	10	13	16	16	100
1952	11	0	7	10	11	11	100
1954	16	2	14	15	16	16	100
1955	8	0	3	3	6	7	88
1956	10	2	6	7	9	9	90
1957	7	1	4	3	2	6	86
1959	9	0	0	0	2	6	67
1960	1	0	1	1	1	1	100
1961	1	0	0	0	0	1	100
1962	6	0	1	1	2	2	33
1963	1	0	1	0	1	1	100
1964	1	0	0	0	0	1	100
1967	2	1	2	2	2	2	100
1968	10	3	10	10	10	10	100
1969	9	0	7	7	8	8	89
1970	10	4	5	6	6	8	80
1972	6	4	5	6	6	8	100
1974	2	1	2	2	2	2	100
1975	5	2	5	5	5	5	100
1976	4	4	4	4	4	4	100
1977	3	2	2	3	3	3	100
1978	19	7	11	12	15	16	84
1981	5	2	4	3	4	5	100
1983	8	2	6	6	7	8	100
1984	2	0	1	1	1	1	50

TABLE 2.—(*Continued*)

No. Selection	No. of rows[c]	No. of rows having smut					Rows smutted, 1943
		1939	1940	1941	1942	1943	
							Per cent
1985	11	0	1	1	1	2	18
1986	24	7	18	20	23	24	100
1987	1	0	0	0	0	0	0
1989	15	0	3	1	3	8	53
1990	6	1	5	6	6	6	100
1991	1	0	1	1	1	1	100
1992	5	3	5	5	5	5	100
1994	13	1	3	8	9	11	85
1995	8	0	0	1	3	6	75
1997	14	6	10	13	13	14	100
1999	12	1	5	10	11	11	92
2004	3	1	2	2	0	2	67
2005	1	1	1	1	1	1	100
2006	9	6	7	7	9	8	89
2007	2	0	1	1	2	2	100
2008	5	1	4	5	5	5	100
2012	7	0	1	5	5	7	100
2019	2	0	1	1	2	2	100
2412	24	2	8	13	18	22	92
Lind selection	14	6	9	9	12	13	93
101	1	0	0	0	1	1	100
102	1	0	1	1	1	1	100
103	1	0	0	1	1	1	100
104–6	1	0	0	1	1	1	100
Totals	690	124	402	460	533	595	.....
Percentage smut	.....	18	58	67	77	86	.....

[a] The plants were transplanted from the greenhouse to the field in the spring of 1937 but were not space-planted. They headed for the first time during the summer of 1937, and since no smut appeared in 1937 and 1938, these years are omitted from the table.

[b] This table was prepared by E. J. Kreizinger, formerly Assistant Agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering.

[c] Each row resulted from seed taken from a single plant selection.

These data constitute strong circumstantial evidence that *Ustilago spegazzinii* and *U. spegazzinii* var. *agrestis* have a unique life history. It seems very probable that the incubation period is at least two years, or, more exactly, that symptoms are not expressed until the third heading after infection, or even later. Also, the yearly increase of stem smut in the stands of slender and crested wheatgrass strongly indicates a plant-to-plant spread of this disease.

EXPERIMENTAL DATA

Results of tests with naturally inoculated seed.—From the plot of slender wheatgrass in which stem smut was severe in 1939, seed was collected during the same season as follows: (1) from smut-free culms on otherwise smutted plants; (2) from smut-free plants with heavily-smutted plants to the windward (prevailing S.W. winds); (3) from smut-free plants entirely surrounded by smutted plants; and, (4) from healthy plants surrounded by

healthy plants. This seed was planted in the greenhouse in wood veneer plant bands and the seedlings transplanted to the field in the spring of 1940. The plants headed well in 1940 and in three successive seasons, but no stem smut appeared in any of them.

Results of artificial inoculations.—During the summer of 1939, mature plants and blossoms were inoculated with *Ustilago spegazzinii* var. *agrestis* and seed was inoculated by the vacuum method. The inoculation of mature plants was as follows: The plants were clipped back (in August, 1939) to within 5 or 6 inches above the ground. They were then sprayed with a spore suspension and covered with several layers of wet burlap for 48 hours. Crested wheatgrass, slender wheatgrass, and Canada wild rye (*Elymus*

TABLE 3.—*Results of blossom, seed, and plant inoculations of Agropyron trachycaulum, A. cristatum, and Elymus canadensis with stem smut, Ustilago spegazzinii var. agrestis. Blossom inoculations performed 1938, seed and plant inoculations, 1939*

Collection and source of inoculum	Seed and blossom inoculations, all species[a]			Plant inoculations								
				Agropyron trachycaulum			*Agropyron cristatum*			*Elymus canadensis*		
	1940	1941	1942	1940	1941	1942	1940	1941	1942	1940	1941	1942
F–C *Agropyron repens*	–[b]	–	–	–	+	+	–	+	+	–	–	+
F–D *A. cristatum*	–	–	–	–	–	+	–	–	+	–	–	+
F–E *A. trachycaulum*	–	–	–	–	–	–	–	+	+	–	–	+
F–G *Elymus condensatus*	–	–	–	–	+	+	–	–	+	–	–	+
F–H *Agropyron spicatum*	–	–	–	–	–	+	–	–	+	–	–	+
F–I *Elymus glaucus*	–	–	–	–	+	+	–	–	–	–	–	+

[a] Since all seed and blossom inoculation results were negative the data are presented in this condensed form.

[b] Only the incidence (+) or absence (–) of stem smut is recorded. Percentages of infection were not determined, but the amount varied from a trace in 1941 to heavy in 1942. For the sake of brevity the check rows are not included but these were maintained for all inoculations and were smut-free.

canadensis L.) were each inoculated with 6 collections of stem smut from different hosts. The inoculated plants headed well in 1940 and subsequent years. No stem smut appeared in 1940; in 1941, however, slight infection was noted in some of the rows of slender wheatgrass and crested wheatgrass. In the summer of 1942 stem smut was abundant in nearly all the rows of slender wheatgrass and crested wheatgrass, and in all the rows of the Canada wild rye. No smut occurred in the check rows at any time.

In the experiment involving blossom inoculation whole spikes were inoculated (during the summer of 1938) with suspensions of stem smut spores, using Moore's portable evacuation method (12). Attention was given to different stages represented in the development of the fertilized ovaries, from the time of pollination to the medium dough stage. The same grasses and

the same smut collections were used as in the plant inoculations. The seed from such inoculated spikes were planted in plant bands and the seedlings transplanted to the field in the spring of 1939. The plants headed well in 1939 and in subsequent years, but no smut appeared at any time.

Also in the spring of 1939, seed of the same grass species was inoculated with the same 6 stem smut collections by immersion in aqueous spore suspensions under partial vacuum. This inoculated seed was planted in the greenhouse and the resulting seedlings were later transplanted to the field. These also headed well in 1939 and in subsequent years, but no smut appeared.

Table 3 shows the success resulting from the plant inoculations and the failure with the seed and blossom inoculations. The results with the plant inoculations substantiate the field observations that stem smut occurs in the third or later heading after planting. The only exception to this that has thus far been encountered is seen in the appearance of a very slight amount of stem smut in *Agropyron trachycaulum* and *A. cristatum* in 1941, in the second heading after plant inoculation in 1939.

It appears that stem smut caused by *Ustilago spegazzinii* and the variety *agrestis* is not seed-borne, either through blossom infection or external seed contamination. Rather, it seems probable that meristematic regions of the plants are infected and that this infection is not expressed by sporulation before the second heading following infection, and usually not until the third and subsequent headings.

DISCUSSION

The foregoing experimental data are probably insufficiently exhaustive to establish that infection of the host by *Ustilago spegazzinii* and the var. *agrestis* occurs in the purely vegetative tissues of the host to the exclusion of other modes of infection. The experiments which seem to establish this important part of the life history need amplification. This is especially needed to determine whether or not some injury to the plants is necessary at the time of inoculation, and also to determine if susceptible plants of any age, from seedling to maturity, can be infected. However, the negative results of the seed and blossom inoculations are in support of the infection of vegetative tissues. Also in support of the latter, is a vast accumulation of observations that indicate the infection of vegetative parts of the host during the period of sporulation of the smut, and the lapse of at least 2 years between time of infection and the appearance of stem smut symptoms.

Observations of the incidence of stem smut in stands of crested wheatgrass, slender wheatgrass, *Elymus spp.*, *Poa spp.* and other grasses during the past several years show an alarming tendency for the disease to "build up" in established stands. This seems entirely in line with the demonstration of the ability of the stem smut organism to infect mature plants. It seems likely that there is a plant-to-plant spread, once the fungus becomes established in a field or other planting. If this should prove to be the case, that stem smut spreads from plant to plant, once established, then the pres-

ence of stem smut would certainly be a factor in determining how long to maintain a stand of a susceptible species, because stem smut certainly renders the affected grass unfit for hay and seed, and possibly for pasture also. The control of such a disease would be very difficult, with the only practical method lying in the use of resistant varieties. Very little resistance has been apparent, however, in the plantings and species that have been under observation.[3] Some salvation may be found in the fact that stem smut has such a long incubation period, and in the fact that apparently the disease is not seed-borne. Thus it seems probable that plantings of susceptible grasses (and most of our locally better species are susceptible) could be maintained five years or more, unless there were a heavy initial infection.

The mode of infection in *Ustilago spegazzinii* and the var. *agrestis*, by the infection of vegetative tissues, is not new to the biology of the smut fungi, but apparently such a long incubation period is unique in this group of plant pathogens. There appears to be no record of any other smut fungus in which sporulation is delayed until at least the second and usually the third heading following infection, after which it is perennial in the perennial parts of the host. At present a plausible explanation for this phenomenon is entirely lacking.

If, as the data indicate, stem smut caused by *Ustilago spegazzinii* and the variety *agrestis* is not seed-borne, the question arises as to the source of infection in the many grass species which have recently been smutted in the Pullman area. In answer to this question an accusing finger may well be pointed at extensive natural stands of quack grass, *Agropyron repens*. Large patches of this grass, very heavily infected with stem smut, are so common locally that it seems quite possible that there could be a sufficiently heavy spore shower to infect other grasses over a wide area. However, it remains for cross-inoculation experiments to definitely prove or disprove this theory. Thus far, the infection of crested wheatgrass (*A. cristatum*), slender wheatgrass (*A. trachycaulum*), and Canada wild rye (*Elymus canadensis*) with stem smut from local *A. repens* is in support of the theory.

SUMMARY

1. The results are given of seed, blossom, and mature plant inoculations with *Ustilago spegazzinii* and *U. spegazzinii* var. *agrestis* (*U. hypodytes* (Schlect.) Fr.), in the Pullman, Washington, region.

2. Infection was obtained in *Agropyron cristatum*, *A. trachycaulum*, and *Elymus canadensis*, with stem smut spores from several species of *Agropyron* and *Elymus* only by inoculation of mature plants.

3. Seed and blossom inoculations gave only negative results. No smut appeared in several hundred plants of *Agropyron trachycaulum* grown from seed taken from partially infected plants and from plants more or less surrounded by heavily infected plants.

[3] In table 2, are listed several selections of crested wheatgrass which appear to have a high degree of resistance to stem smut. These are being used by E. J. Kreizinger as a source of stem smut resistance in crested wheatgrass.

4. The period of incubation was at least 2 years, and more often 3 years. In the successful inoculation experiments a trace of stem smut appeared in the second heading following inoculation, but at the third heading it was abundant. In numerous observations of the incidence of stem smut in commercial plantings, seed increase plots, and nursery rows the stand in every case was in at least its third heading.

5. It is thought that the source of infection lies in the common local heavy infestations of stem smut in quack grass, *Agropyron repens*. Experimentally, stem smut was induced in *A. cristatum*, *A. trachycaulum*, and *Elymus canadensis* with spores from *A. repens* as inoculum.

6. It has been observed that stem smut, once established in a stand of a susceptible grass, tends to increase rapidly in successive years, thus indicating a plant-to-plant spread.

7. Control of such a disease promises to be difficult. Probably the most practicable method will be the use of resistant strains of forage grasses. It is suggested, also, that because of the long incubation period, stem smut will not appear until at least 3 years after a susceptible grass is planted and will probably not become much of a production factor for 2 or 3 years more (unless there is a heavy initial infection), after which it may be advisable to plow up the stand.

WASHINGTON AGRICULTURAL EXPERIMENT STATION,
PULLMAN, WASHINGTON.

LITERATURE CITED

1. BERKELEY, M. J. Introduction to Cryptogamic Botany. 604 pp. London. 1857.
2. BOND, T. E. T. Observations on the disease of sea lymegrass (*Elymus arenarius* L.) caused by *Ustilago hypodytes* (Schlecht.) Fries. Ann. Appl. Biol. **27**: 330–37. 1940.
3. BORNHÖVD, LISELOTTE. Beitrage zur Biologie von *Ustilago hypodytes* (Schldl.) Fr. auf *Elymus arenarius* L. Phytopath. Zeitschr. **9**: 69–97. 1936.
4. BOSS, GEORG. Beitrage zur Zytologie der Ustilagineen. Planta **3**: 597–627. 1927.
5. CIFERRI, RAPHAEL. Flora Italica Cryptogama, Pars. I: Fungi, Ustilaginales. Fasc. 17, 443 pp. 1938.
6. CLINTON, G. P. Ustilaginales. North American Flora **7**: 1–82. 1906.
7. FEUCHT, W. Morphologische Abweichungen an *Bromus erectus*, verursacht durch *Ustilago hypodytes*. Angewandte Botanik **12**: 1–16. 1930.
8. FISCHER, GEORGE, W. Some new grass smut records from the Pacific Northwest. Mycologia **30**: 385–395. 1938.
9. ————and ELISA HIRSCHHORN. A critical study of some species of *Ustilago* causing stem smut on various grasses. Mycologia **37**: 236–266. 1945.
10. KHARBUSH, S. S. Conrtibution a l'étude des phenomenes sexuels chez les Ustilaginées. Ann. Sci. Nat. Bot. Ser. X, **9**: 285–297. 1927.
11. LIRO, J. I. Die Ustilagineen Finnlands. Ann. Acad. Sci. Fenn., Ser. A., **17**: 1–636. 1924.
12. MOORE, M. B. A method for inoculating wheat and barley with loose smuts. Phytopath. **26**: 397–400. 1936.
13. ZUNDEL, GEORGE L. Ustilaginales. Additions and corrections. North. Amer. Flora **7**: 971–1045. 1939.

THE REACTION OF 21 SPECIES IN THE CUCURBITACEAE TO ARTIFICIAL INFECTION WITH CANTALOUPE POWDERY MILDEW (ERYSIPHE CICHORACEARUM DC.)

THOMAS W. WHITAKER[1] AND DEAN E. PRYOR[2]

(Accepted for publication February 17, 1945)

Powdery mildew (*Erysiphe cichoracearum* DC.) has been a serious menace to the cantaloupe industry in practically all of the producing regions of the southwestern States since 1926. In developing a plant breeding program to combat this parasite by means of disease-resistant varieties, it is important to know the reactions of other species in the Cucurbitaceae to artificial infection with the fungus. This information should indicate possible potential hosts in the native, as well as the cultivated, flora. It may perhaps furnish a valuable clue in pointing out those genera and species that carry genes for disease resistance and, hence, are of possible value for hybridization with *Cucumis melo* L., the species to which the cantaloupe and related melons belong.

While this survey was by no means extensive, it included representatives of many of the genera of the Cucurbitaceae that have been cultivated from time to time; also, with one exception, it included the genera of the Cucurbitaceae native in southern California.

METHODS

Plants grown in the greenhouse were artificially inoculated in a chamber designed for the purpose.[3] Within 16 days reasonably precise readings could be made of the resistance of individual plants. For convenience in recording results, 5 categories based on the extent of mycelium growth and degree of sporulation on leaves, cotyledons, and stems were used: 0 indicated no macroscopically visible mycelium and 4 indicated abundant mycelial growth and conidial production (See Pryor and Whitaker[4] for details regarding rating the amount of mildew).

RESULTS

The reactions of 21 species in 11 genera are recorded in table 1. Seven of the 21 species had a high level of resistance to cantaloupe powdery mildew: *Citrullus vulgaris* Schrad. (watermelon), *Cucumis anguria* L. (West Indian gherkin), *Cyclanthera explodens* Naud., *C. pedata* Schrad., *Ecballium elaterium* (L.) A. Rich. (squirting cucumber), *Luffa acutangula* (L.) Roxb. (dishrag gourd), and *L. aegyptiaca* Mill. (dishrag gourd).

Three native species were susceptible: *Cucurbita foetidissima* H.B.K.

[1] Geneticist, United States Department of Agriculture.

[2] Associate Plant Pathologist—on military leave.

[3] Pryor, Dean E. The influence of vitamin B_1 on the development of cantaloupe powdery mildew. Phytopath. 32: 885–895. 1942.

[4] Pryor, D. E., and Whitaker, T. W. The reaction of cantaloupe strains to powdery mildew. Phytopath. 32: 995–1004. 1942.

TABLE 1.—*The reaction of species in the Cucurbitaceae to artificial infection with cantaloupe powdery mildew*

Species	No. plants tested	Mildew reaction[b]		
		Leaves	Cotyledons	Stems
Benincasa hispida (Thumb.) Cogn.	21	0	0–2	0–4
Bryonia dioica L.	9	0–2	0–4	0
Citrullus vulgaris Schrad.	12	0	0	0
Cucumis melo L.	100[a]	0–4	0–4	0–4
Cucumis sativus L.	100[a]	1–4	3–4	0–4
Cucumis anguria L.	4	0	0	0–2
Cucumis metuliferus E. Mey.	3	4	1–2	4
Cucurbita pepo L.	100[a]	2–4	2–4	0–4
Cucurbita moschata Duch.	100[a]	1–4	2–4	0–4
Cucurbita maxima Duch.	100[a]	0–4	2–4	0–4
Cucurbita ficiolia Bouché	12	0–3	2–4	0
Cucurbita palmata, S. Wats.	4	3–4	4	1–4
Cucurbita foetidissima H. B. K.	12	3–4	4	1–4
Cyclanthera explodens Naud.	6	0	2	0–1
Cyclanthera pedata Schrad.	9	0–2	0–2	4
Ecballium elaterium (L.) A. Rich.	10	0–1	0?	0
Echinocystis macrocarpa Greene	6	0–2	–	0–2
Lagenaria leucantha (Duch.) Rusby	24	3–4	4	3–4
Luffa acutangula (L.) Roxb.	3	0	0	0
Luffa aegyptiaca Mill.	6	0	0	0
Trichosanthes anguina L.	20	0–1	0–4	0–4

[a] 100 plants or more.

[b] The mildew reaction often is the range found between plants, indicating, in the case 0–4, that some plants were completely immune while others were highly susceptible. Thus in some species there is considerable heterozygosity insofar as resistance to powdery mildew is concerned.

(wild gourd), *C. palmata* S. Wats. (coyote melon), and *Echinocystis macrocarpa* Greene (Chilicothe mock cucumber). We have found powdery mildew on plants of *Cucurbita foetidissima* and *C. palmata* growing in the wild. This indicates that the susceptible native flora may be a source of the fungus for infecting cultivated plants.

No species closely related to *Cucumis melo* was found with resistance of a higher order than that found in *C. melo* itself. Those genera and species having virtual immunity from powdery mildew are probably all too distantly related to be of value in attempted hybridization with *C. melo*. The failure to find resistant individuals in a species is no proof of complete susceptibility in that species. Had greater numbers of plants been tested, some resistant individuals might be expected because of the heterozygosity of many of these species. The finding of resistance, however, is definite, since the possibility of escape from infection under the conditions imposed is extremely remote.

UNITED STATES HORTICULTURAL FIELD STATION,
LA JOLLA, CALIFORNIA.

TECHNIQUE FOR HASTENING FOLIAGE SYMPTOMS OF PSOROSIS OF CITRUS[1]

JAMES M. WALLACE[2]

(Accepted for publication March 8, 1945)

INTRODUCTION

The symptoms of the psorosis virus diseases of citrus have been described recently by Fawcett and Bitancourt (4). As pointed out by these authors, the young-leaf symptoms are common to several related viruses, or forms of the same virus, affecting citrus. Although the young-leaf symptoms of psorosis are sometimes very conspicuous on diseased trees in the field, they are so variable and so difficult to detect under some conditions that their absence does not necessarily prove that the tree is free of the virus. Furthermore, the scaling or bark-lesion symptom (4) of psorosis commonly does not develop on orchard trees until the trees are from 12 to 16 years old, or older, even though the trees have been systemically infected throughout this time, having been propagated from diseased buds.

Fawcett (1, 2, 3) demonstrated that the buds and bark of psorosis-affected trees contain virus, and that propagation from diseased trees is the only important means of increase and dissemination of psorosis throughout the citrus-producing regions of California.

Fawcett and Cochran (5) were able to infect healthy trees by grafting to them small pieces of bark from diseased trees. Their method consisted of removing a small disk of bark from the tree to be inoculated and replacing it with a disk of equal size from a diseased tree. The bark disks were removed with a cork-borer, and the completed graft was wrapped with grafting cloth. These writers showed further that scaling could be induced on inoculated trees in less than 5 months if bark patches were transplanted directly from lesion areas to healthy trees. On the other hand, if the patches were taken from normal bark 3 to 6 inches away from the lesions, no bark symptoms had developed on inoculated trees within 3 years. This suggested that a long time would elapse before bark symptoms would develop on such experimentally infected trees, as is the case when trees are propagated from normal-appearing buds of diseased trees.

The transmission studies of Fawcett (1, 2, 3) and of Fawcett and Cochran (5) consisted primarily of inoculations of field and nursery trees. No particular study was made of the time required for symptoms to develop on the young leaves of experimentally infected trees, but Fawcett observed such symptoms in 5 weeks on shoots that developed from diseased buds placed in healthy trees.

In 1942 the writer began some investigations on this group of virus dis-

[1] Paper No. 527, University of California Citrus Experiment Station, Riverside, California.

[2] Associate Plant Pathologist in the Experiment Station, University of California.

eases of citrus. One of the first studies was for the development of a standardized inoculation technique for use with these viruses, and, particularly, for decreasing the time required for leaf symptoms to appear. A useful inoculation technique has evolved that commonly induces leaf symptoms on young trees within 4 weeks after inoculation, and occasionally in as short a time as 15 days.

INOCULATION METHODS

In order to make possible the use of large numbers of plants in greenhouse studies, and to shorten the time required to grow the experimental plants, the present study dealt chiefly with seedling orange trees having a basal trunk diameter of approximately 5 to 8 mm. By seeding in beds under lath and later transplanting individual seedlings to 6-inch pots, it was pos-

FIG. 1. Bark-patch inoculations of seedling orange trees. A. Stock tree prepared for bark graft, showing peeled bark strip, with leaf attached. B. Bark patch (inoculum) in place on stock tree. C. Completed bark graft held in place by rubber tape.

sible in 5 or 6 months to grow trees suitable for inoculation by means of bark grafts. For most of these studies, Homosassa sweet-orange seedlings were used.

The small trunk diameters of the seedlings made it necessary to use rectangular pieces of bark instead of round disks for graft-inoculation of the trees. A location on the basal portion of the seedling trunk, free of thorns or leaf scars, is selected, and the bark is stripped downward after making one horizontal and two longitudinal cuts through the bark with a razor blade. When possible, the point selected for the bark graft is immediately below and in line with an attached leaf (Fig. 1). The horizontal cut is made in the bark above and close to the leaf petiole. The longitudinal cuts extend downward for about an inch, and the distance between these cuts is determined by the circumference of the tree and the size of the bark patch to be inserted. By using the leaf at the top as a "handle," the bark can

usually be separated from the wood by a slight outward pull, the leaf remaining attached at the top of the strip of peeled bark. When the bark does not peel easily or does not loosen easily at the crosscut, a slight pressure at that point with the blade of a knife or scalpel usually loosens it sufficiently so that it can be peeled the remaining distance with the fingers.

The bark to be inserted as inoculum is taken usually from small twigs or limbs of diseased trees, and the area from which it is to be obtained is scraped very lightly with a razor blade to remove the cuticle and epidermal cells. Two horizontal and two longitudinal cuts are then made so as to have a rectangular piece of bark only slightly smaller than the peeled area of the tree to be inoculated. This bark is then pried loose at one of the cut ends, stripped from the wood, and placed in position in the seedling tree, its cambium adjacent to the cambium of the tree. The peeled strip of bark is then folded back in position over the inserted bark patch. If the leaf is still attached to the upper end of the bark to be folded back, it can be held against the trunk with the fingers of one hand while the grafting tape is applied over the completed bark graft with the other. Electrician's splicing compound was found to be excellent for this type of graft. This material is a rubber tape, one side of which is self-adhering. With the inoculation patch in place and the bark strip folded back over it, a small piece of tape is placed over the bark patch and the two ends are pressed together tightly on the side of the tree opposite the bark patch. The inoculated trees are topped a few inches above the bark patch.

Experiments in which the seedling trees were cut back, as in figure 1, in comparison with those in which defoliation or other treatments were employed to speed virus invasion and symptom development, demonstrated that topping gave the best results. Regardless of the growth condition of the trees at time of topping, this treatment stimulated early growth of axillary buds, and when growth was rapid, the leaves developing on the forced shoots sometimes showed symptoms within 15 days after inoculation.

SYMPTOMATOLOGY

The symptoms produced on trees inoculated in the manner just described are variable and seem to be affected by numerous factors. The present paper deals chiefly with the initial symptoms on the young shoots that develop from axillary buds of the topped trees after inoculation. For convenience of description, the symptoms are classified as (a) typical young-leaf symptoms and (b) shock symptoms. One type, or both types, may be induced, the form dependent upon the source of the inoculum.

Typical Young-Leaf Symptoms. Bark-patch inoculations, with bark from infected trees showing neither bark nor twig symptoms, or with normal-appearing bark from trees on which some bark lesions are present, sometimes result only in flecking and stippling of the newly formed leaves (2, 4). Such symptoms are rather typical of those commonly observed on young leaves in the field. Usually, however, the leaves show more chlorotic blotching or

spotting, and frequently some of the affected leaves curve downward. In the greenhouse, the young-leaf symptoms are retained for a longer time than in the field, and, in some cases, as the leaves mature they may show light-colored spots or areas which are not the same as the mature-leaf symptoms described by Fawcett and Bitancourt (4). The young leaves of the succeeding flushes of growth may or may not have symptoms. When such symptoms do reappear, they are similar to those on field trees, and, as on field trees, symptoms occur on only some of the leaves.

FIG. 2. A. Seedling orange tree with shock symptoms following bark-patch inoculation with psorosis virus. Note bending of shoots and loss of leaves on shoots arising in line with bark patch. B. Healthy control tree grafted with virus-free bark patch. Both trees photographed 21 days after grafts were made. All shoot growth developed after trees were topped.

Trees having these symptoms are not noticeably retarded in growth and except for the leaf symptoms are comparable to healthy controls, remaining so unless mature-leaf, bark, or twig symptoms develop on them.

Shock Symptoms. Under certain conditions not yet well understood, orange seedlings infected with psorosis virus by the inoculation methods described herein have shown severe injury on some of the shoots and leaves that develop immediately after the trees are topped. Because such symptoms have not been induced by re-inoculation or by topping of trees previously infected and invaded by the psorosis virus, this reaction is described

as a "shock" stage of the disease. The first indication of this severe type of symptom is that some of the newly developing shoots begin to twist slightly, and as growth continues the shoot becomes bowed or curved. The leaves that have formed also curve downward and drop upon the least disturbance. The tiny growing-point leaves wither and drop, necrotic spots sometimes appear on the leaves and the young stems, and at later stages the shoots die back partly or completely to the main trunk. The dead, twisted shoots dry and darken and frequently remain attached to the tree for a long time.

In figure 2, the two upper shoots of tree A show the bending and loss of outer leaves typical of a tree in the shock stage. The short shoot, fourth from the top, is completely defoliated. Tree B is a control plant which received a virus-free bark patch. As a rule, only those shoots arising in line with the bark patch will show the shock reaction. Commonly, the shoots on the opposite side will appear normal, although at later stages they may have only typical leaf symptoms.

The shock symptoms have been obtained only with the bark-scaling varieties of psorosis, psorosis A and psorosis B, and are not always induced by inoculations with these two varieties of the virus. So far, the results indicate that with certain strains of the psorosis-A virus, the shock stage of symptoms is induced by bark-patch inoculations irrespective of whether the inoculum is from normal-appearing bark or directly from psorosis bark lesions. The severe shock reaction has resulted in numerous instances when the bark patch used as inoculum was from trees that had no bark, twig, or mature-leaf symptoms. Thus, the shock reaction, occurring within 2 to 4 weeks on the newly developing shoots of small seedling trees that are cut back when the bark graft (inoculation) is made, does not appear to be influenced by the source of inoculum, that is, whether it is from lesion or non-lesion bark. On the other hand, such secondary symptoms as twig and mature-leaf symptoms have developed only on trees of these experiments that were infected from bark from lesion areas of diseased trees. On such trees the twig and mature-leaf symptoms have been observed in some instances within 42 days after the inoculation grafts were made.

In six experiments including 105 infected trees on which records were taken 21, 24, and 28 days after inoculation, 82 plants developed symptoms within 21 days or less. Within 28 days, symptoms had appeared on all but three plants. In many tests all inoculated trees became infected but on some occasions the bark patches decayed and no tissue union resulted. Fewer failures of transmission result when the outer bark of the bark patches is scraped slightly to remove surface dirt and organisms. This is particularly desirable when bark from older twigs and limbs is used for inoculum. Even when the bark patches are from young, green twigs, removal of the cuticle and epidermis is of some benefit in that more surface for callus growth and union is provided.

SUMMARY AND CONCLUSIONS

Inoculation of young sweet-orange seedlings having a basal trunk diameter as small as 5 mm. has provided a method of inducing symptoms of the

psorosis diseases of citrus in a short time. Small bark patches from diseased trees, or from trees to be tested, are placed against the cambium beneath strips of bark peeled back for this purpose on the trees to be inoculated. Callus forms rapidly and the virus is transmitted. The small stock trees are topped to within 2 or 3 inches of the bark patch.

Psorosis symptoms, either of a severe shock type or of the more or less typical young-leaf stippling and blotching usually appear on the growth arising from the upper axillary buds after the trees are topped. In some instances, inoculated trees have no symptoms during the first cycle of growth after topping, symptoms appearing at later cycles. This probably results from a delayed movement of the virus into the growing point tissues and its failure to invade the newly forming leaves early enough in their development to induce symptoms. Shock symptoms have developed only on the forced growth of topped trees not systemically invaded by the virus.

Inoculated trees that develop the severe shock stage of symptoms during the first flush of growth commonly have only typical young-leaf symptoms in later flushes and, except for lessened growth during the early shock stage, do not differ from the diseased trees that never showed shock injury. If the bark patches used for inoculum are taken directly from lesion areas, the infected stock trees develop bark and mature-leaf symptoms early, often in less than 2 months, and the trees are permanently retarded in growth.

Inoculation procedure that lessens the time required for symptom development of psorosis-affected orange trees is of value in providing a short-time method for transmission studies. With such technique it is now possible to conduct large-scale fundamental investigations of the psorosis virus diseases, which, because of their peculiarities and their somewhat unpredictable behavior in the field, have yielded slowly to experimental study.

An additional feature of these inoculation methods lies in their use in connection with the registration of virus-free parent trees for propagation of nursery stock. In place of the present practice of periodic inspection of trees proposed as bud-wood parents, and the somewhat delayed and not too satisfactory field-progeny tests from such trees, the inoculation methods described here give promise of providing a short-time test for presence or absence of psorosis virus in trees selected by nurserymen and propagators as bud-wood parents. Although further studies may show that the different, known varieties of the psorosis virus—namely, psorosis A, psorosis B, concave gum, blind pocket, and crinkle leaf, and possibly strains of these varieties—may differ in symptomatology, present indications are that if present in the tissue used for inoculum, any or all of them will induce some type of detectable symptom on the first or second flush of growth of the inoculated trees.

Additional study must be completed of such factors as virus strains, virus concentration, and type of tissue used for graft-inoculation, in relation to symptomatology of psorosis, before it can be determined whether this method can be substituted completely for the present methods of certifying parent

trees. At present this inoculation technique is being used to supplement the regular inspection method of parent-tree certification and has already been of value in eliminating some trees that have been considered for registration. In other instances, this method has provided positive substantiation of the presence of psorosis in certain trees suspected of being diseased, but on which reliable symptoms had not been found in the field. If further investigations show that negative tests by this method can be relied upon as proof that the tree under consideration is free of psorosis virus, it may be possible to use this method of indexing almost exclusively in the registration of disease-free trees for bud-wood parent trees.

UNIVERSITY OF CALIFORNIA,
CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA.

LITERATURE CITED

1. FAWCETT, H. S. New symptoms of psorosis, indicating a virus disease of citrus. (Abstr.) Phytopath. 23: 930. 1933.
2. ————. Is psorosis of citrus a virus disease? Phytopath. 24: 659–668. 1934.
3. ————. Transmission of psorosis of citrus. (Abstr.) Phytopath. 28: 669. 1938.
4. ————, and A. A. BITANCOURT. Comparative symptomatology of psorosis varieties on citrus in California. Phytopath. 33: 837–864. 1943.
5. ————, and L. C. COCHRAN. Symptom expression in psorosis of citrus as related to kind of inoculum. (Abstr.) Phytopath. 32: 22. 1942.

THE INVASION OF THE INTERNAL STRUCTURE OF COTTON SEED BY CERTAIN FUSARIA[1]

B. A. Rudolph[2] and G. J. Harrison[3]

(Accepted for publication March 12, 1945)

Experiments recently reported by the writers of this paper (10) show that cotton seed is an exceedingly insignificant factor in the dissemination of Verticillium wilt in California, if indeed, it is a factor at all. In one phase of the experiments to determine whether the internal structure of the seed may be parasitized by the fungus, cultures were made from 3371 mature cotton bolls produced on plants severely affected with the disease. Of these, the fungus succeeded in reaching the receptacles of only 150 (4.44 per cent) and penetrated as far as the bases of the placental columns of only 2 (0.00059 per cent). It never succeeded in reaching the placentae or funiculi, much less the seeds.

Several species of *Fusarium* were isolated from time to time, not only from the roots, stems, receptacles and bases of placental columns, but also from those tissues which *Verticillium* apparently had been unable to reach, namely, the placentae, funiculi, and seeds.

A number of workers have already reported isolating *Fusarium* from the interior of cotton seed; Hansford (8) isolated *F. moniliforme* Sheld. from as much as 25 per cent of the seed with which he worked. He also isolated two species of *Fusarium* of the section *Elegans* (7). Similarly, Elliott (2) reported isolating 1, and Crawford (1) 3 unidentified species of *Fusarium* from within the testa. Not all of these writers expressed opinions as to how the fungus reached the inside of the seed, but in some instances the inference is that it got there by way of the vascular system. Hansford (8) particularly makes it clear that the seed with which he worked was from bolls that showed neither external nor internal evidence of disease, and he was at a loss to explain the presence of the fungus within the seed.

Elliott (2, 3) and Crawford (1) presented seemingly irrefutable evidence that *Fusarium vasinfectum* Atk. can, and at times does, invade the internal structure of cotton seed and, because of this, they concluded that the disease is seedborne. Such a conclusion is not valid, because of the possibilities that the fungus may (a) die in the seed before it is planted in the spring, thus leaving it as healthy as any other; (b) kill the embryo, thus preventing the production of a plant (Taubenhaus and Ezekiel (11) found this to be true to some extent); or (c) not be able to attack the seedling from its position within the seed, assuming that the fungus is capable of living until planting time in the spring and that it does not kill the embryo. Regardless of whether the embryo be killed, if the fungus can live over in the seed,

[1] Much of the work reported in this paper was done under W.P.A. projects 50–1859, 50–11992 and 50–12456–D.

[2] Associate Plant Pathologist in the California Agricultural Experiment Station.

[3] Agronomist, United States Department of Agriculture.

there is still the possibility that it can establish itself in the soil when such seed is planted.

Neal (9), Gilbert (6) and Fahmy (5) deny that Fusarium wilt is seedborne and base their contention on the fact that, in their experiments, seed taken from diseased plants produced healthy plants when planted the following spring. They did not question, however, whether some of the seed might have once contained the fungus in a living condition. Ezekiel (4, 11) and Taubenhaus (11), on the other hand, not only isolated *Fusarium vasinfectum* from seed taken from diseased plants but also secured diseased plants when they planted the seed under controlled conditions.

Elliott (3) and Crawford (1) have shown that the incidence of internal infection in seed is not great, also that the viability of the fungus may be expected to decline sharply three months after the seed is harvested. Thus, by planting time, the seed containing the viable fungus may constitute a very small percentage of the total and might very easily be missed when limited numbers of cultures are made.

The sharp disagreement existing in the literature today as to whether Fusarium wilt is seedborne stems largely, on the one hand, from the invalid conclusion by certain workers that the mere presence of the fungus within the seed constitutes proof that the disease is seedborne and from the equally invalid conclusion by certain other workers that it is not seedborne because seed from diseased plants usually yields healthy plants when planted the following spring. Apparently time and incidence factors, overlooked by many workers, are involved, as the works of Taubenhaus (11), Ezekiel (4, 11), and Elliott (3) seem to show. The incidence of infection in seed is lower in some years than in others, and there is a tendency for the fungus to die out rapidly after a time in the seed. Also, a certain amount of the seed is killed by the fungus.

SEED STUDIES IN CALIFORNIA

The technique employed in the experiments which showed that internal infection of cotton seed by *Verticillium albo-atrum* rarely if ever occurs in California has been described in considerable detail (10). Cultures were made in the approved, conventional manner. The results, however, were considerably different from those anticipated: whereas *Verticillium* seemingly was unable to reach the placentae, several species of *Fusarium*, which, with the *Verticillium*, inhabited the xylem of roots and stems although its presence there was not suspected, frequently reached the placentae. Table 1 shows the percentage of bolls, the placentae of which yielded Fusaria in culture in 1935 and 1936. The bolls were fully mature but unopened and, aside from being more or less stunted, as they are when the plants have Verticillium wilt, they had no disease symptoms whatever, either externally or internally.

Since the only symptoms of the plants from which the bolls were taken were those ordinarily associated with Verticillium wilt, a question arose as

to whether any of the several Fusaria associated with *Verticillium* in the plants might be an active parasite, capable of attacking cotton seedlings *per se* and producing definite symptoms of its own. To prove this point, cotton seed produced by cotton plants to all appearances healthy at the U. S. Cotton Field Station at Shafter, California, was planted in sterilized pots and large wooden boxes in the greenhouse at the Deciduous Fruit Field Station at San Jose. The soil was sterilized in a steam pressure cooker at a local cannery at temperatures varying from 250° to 268° F for 9 to 18 hours, the lower the temperature the longer the time allowed for sterilization. The seed was delinted with concentrated sulphuric acid, washed, dried, and then examined under the microscope for the tiny, bristle-like hairs that often persist at the stem end of a seed, even though the testa itself may have been charred almost to destruction by the acid. These bristles, when present, were scraped off with a scalpel and the seed was surface-sterilized by immersion in $HgCl_2$, 1/1000, for 5 minutes. It was then washed with sterile water and planted.

TABLE 1.—*Isolations of Fusaria made from the placentae in bolls of cotton plants known to be affected with Verticillium wilt*

Year	Diseased plants	Bolls cultured	Bolls with placentae infected with *Fusarium*	
	No.	*No.*	*No.*	*Per cent*
1935	12	61	5	8.19
1936	53	434	32	7.37

When the young plants were about 6 inches high, the soil of a given lot of pots or boxes was inoculated with homogeneous cultures of one of the several species of *Fusarium* originally isolated from the internal tissues of cotton seed, a second lot with another species, a third with another, and so on. The inoculations were made as follows: A sterile steel bar 1 inch in diameter was pressed into the moist soil to a depth of 6 inches immediately alongside each of the plants. Then a fresh 10-cc maximal slant culture of the fungus, growing in a standard culture tube $\frac{3}{4}$ inch in diameter and 6 inches long, on 2 per cent Czapeck's solution agar, was scraped into each hole. The hole was then pinched shut. Check plants were similarly treated except that inoculum was not placed in the holes.

Cotton does not grow well in the close confinement of pots or even large boxes. The plants were small, but none developed disease symptoms of any kind. In the steles of occasional plants were faint brownish streaks, but there was no relation between the discolorations observed and the results obtained with cultures.

Cultures were made of tissue taken from the steles of all plants at least 6 inches above soil level, on Czapeck's solution agar slightly acidified with lactic acid to suppress bacterial growth. At least 4 months had elapsed since the soil had been inoculated. The plants were fully mature and had produced bolls. The results obtained in three different years are in table 2.

TABLE 2.—*Results of cultures made from the steles of cotton plants grown in steam sterilized soil infested with Fusaria isolated from seed*

Year	Plants grown in soil infested with *Fusarium*	Check plants	Plants infected with *Fusarium*	
	No.	*No.*	*No.*	*Per cent*
1937	60	.....	33	55.00
		29	5	17.44
1938	46	.....	19	41.30
		18	0	0.0
1941	32	.....	7	21.87
		6	4	66.66

It was perfectly clear from the results obtained in 1937 that the so-called healthy seed from which the plants were grown must have harbored the Fusaria internally; the severe preliminary treatment given the seed, consisting of delinting with acid, hand scraping, and disinfecting, precluded all possibility of the fungus being carried on the exterior. Yet both the inoculated and check plants yielded the same species of *Fusarium* abundantly in culture. Not infrequently plants yielded one or more additional species of *Fusarium* other than the one used to inoculate the sterile soil of a given lot of pots or boxes. Such species could have come from only one possible source, namely, the so-called healthy seed from which the plants were grown. And internal infections of such seed must necessarily have taken place in the field by way of the vascular system, since the bolls showed no internal or external evidence of disease whatever at the time the seed was harvested. Original infection of the plant takes place through the roots, but no lesions or other symptoms were on the roots at the time cultures were made from the steles. In 1939 the percentage of infection in the checks actually exceeded that in the plants grown in infested soil. Apparently 1937 was not favorable to natural infection in the field, because the check plants raised in 1938 from seed produced in 1937 were not infected.

Additional evidence that the Fusaria inhabit the interior of the seed was obtained. Seed taken exclusively from bolls the receptacles of which had yielded Fusaria in culture was delinted with concentrated sulphuric acid, scraped free of bristles, disinfected and washed, and planted in the field at the Deciduous Fruit Field Station where cotton had never grown before. Checks were provided, but the seed used was so-called healthy seed

TABLE 3.—*Results of cultures made from the steles of field-grown cotton raised from seed taken exclusively from bolls the receptacles of which had yielded Fusaria in culture*

Material cultured	Plants infected with *Fusarium*	
	No.	*Per cent*
104 plants raised from seed taken from bolls with receptacles infected with *Fusarium*	26	25.00
111 plants raised from healthy seed. Check	22	19.81

obtained from the Shafter Station. Both lots of plants were of equal vigor and without disease symptoms of any kind.

When the plants were 5 months old, cultures were made from stele tissue taken at least 6 inches above ground (Table 3).

The percentage of plants infected with Fusaria was higher in the lot raised from seed taken from bolls the receptacles of which had yielded the fungus in culture; but it was also high in plants raised from so-called healthy seed, which indicates that the fungus was present in much of the seed at the time it was planted. A certain amount of infection may have taken place, of course, in the soil which was not sterile and which may have harbored the Fusaria, but both lots of plants were equally exposed.

Internal Infection of the Seed. To make sure that the Fusaria may penetrate the interior of the seed by way of the vascular system, seed from bolls the receptacles of which had yielded Fusaria in culture was saved, acid delinted, scraped free of bristles, surface-sterilized, washed, and cul-

TABLE 4.—*Results of cultures of cotton seed from bolls the receptacles of which had yielded Fusaria in culture*

Year	Bolls with infected receptacles	Seed in bolls	Seed infected with *Fusarium*	
	No.	*No.*	*No.*	*Per cent*
1938	10	277	72	25.99
1939	19	537	113	21.04

tured. Each seed was cut transversely into halves with sterile instruments and planted in Petri plates of slightly acidified Czapeck's solution agar. To maintain the identity of each seed, the halves were planted in proper sequence on either side of a line drawn across the bottom of each plate with a wax pencil, the funicular halves on one side and the micropilar on the other. Not more than 4 seeds (8 halves) were planted to a plate. More than $\frac{1}{5}$ of the seed was infected internally in 1939 and $\frac{1}{4}$ in 1938 (Table 4).

Field Inoculations. Because cotton grows poorly in pots, the greenhouse studies (Table 2) were repeated in the field at both the San Jose and the Shafter stations. Inoculations were made in the spring when the plants were 6 inches high, and checks were provided. The seed used for planting at San Jose was acid delinted, hand scraped, disinfected, and washed; that used at Shafter was treated with Ceresan at the rate of 3 oz. per 32 lb. of seed. Cultures were made from the steles at least 6 inches above ground level in the late fall or early winter when the plants were fully mature and carrying many bolls. The results are in table 5.

At Shafter and at San Jose all plants grew normally in the field and had no disease symptoms of any kind. Again, as in the case of the pot experiments (Table 2), the percentage of infection in the check plants was high, in 2 instances actually higher than that of the plants grown in infested soil. The reason for this is not clear.

Identification of the Fusaria.[4] The experiments reported here were conducted entirely as a side line in connection with a major project to determine whether Verticillium wilt is seedborne. Time did not permit a detailed study of the many cultures of *Fusarium* isolated from receptacles, placentae, and seed. Moreover, when it was recognized as the result of inoculation experiments that these Fusaria were entirely innocuous and seemingly natural concomitants of healthy plants and seed, there seemed to be no occasion for more precise studies. Since the Fusaria discussed here presumably inhabit the xylem of cotton plants simply as saprophytes, or at most as very weak parasites, the invaded plants and seed, in a broad sense, can be regarded as being as healthy as those in which these fungi do not occur. This view is not extreme. Somewhat similar, but not entirely analogous,

TABLE 5.—*Results of cultures made from field-grown cotton plants produced from healthy seed and grown in soil infested with Fusaria*

Locality and year	Plants grown in soil infested with *Fusarium*	Check plants	Plants infected with *Fusarium*	
	No.	*No.*	*No.*	*Per cent*
San Jose, 1940	136	.....	6	4.41
	.....	13	2	15.38
San Jose, 1941	115	.....	51	44.34
	.....	30	12	40.00
Shafter, 1941	483	.....	88	18.21
	.....	104	22	21.15

instances can be found in the higher animals, including man, whose lower intestines swarm with saprophytic organisms. Such a situation is general, and the individual is not considered diseased because of their presence.

Among the Fusaria isolated from xylem in all parts of the plants and from the seed, and used in the inoculation experiments, were *Fusarium moniliforme* Sheld., *F. oxysporum* Schl., and *F. scirpi* Wollenw. All three have been reported on cotton. In addition there was another species, the identity of which was never determined. Unfortunately a culture set aside for study became contaminated and died.

With the exception of *Fusarium scirpi*, none of the species appeared to injure the seed. *F. scirpi* not infrequently was isolated from immature seed that had broken down with a soft, brownish decay. The bolls themselves were to all appearances healthy. Yet it is evident that this fungus does not always kill the seed, since plants grown from acid-delinted seed in sterile soil often yielded *F. scirpi* when cultured. It was distinctly less frequently isolated, however, from either plant stems or seed than were the other species.

SUMMARY

Several species of *Fusarium* appear to be innocuous concomitants of healthy cotton seed. They reach the seed by way of the xylem which they

[4] Grateful acknowledgment is made to Dr. W. C. Snyder for his invaluable assistance in identifying the Fusaria.

seemingly inhabit as saprophytes, since the plants have no disease symptoms whatever. Two have been identified as *F. moniliforme* Sheld. and *F. oxysporum* Wollenw. A third, *F. scirpi,* is capable at times of producing a soft, brown rot of immature seeds. A fourth species remains unidentified. Had time permitted more detailed study, additional species or strains might have been recognized.

UNIVERSITY OF CALIFORNIA DECIDUOUS FRUIT FIELD STATION,
SAN JOSE, CALIFORNIA,
AND
U. S. COTTON FIELD STATION,
SHAFTER, CALIFORNIA.

LITERATURE CITED

1. CRAWFORD, R. F. Fungi from the interior of cotton seed. Phytopath. **13**: 501–503. 1923.
2. ELLIOTT, JOHN A. Cotton wilt a seed-borne disease. (Abs.) Phytopath. **12**: 50–51. 1922.
3. ———. Cotton-wilt, a seed-borne disease. Jour. Agr. Res. [U.S.] **23**: 387–393. 1923.
4. EZEKIEL, WALTER N. Transmission of diseases on and in cotton seed. Intntl. Crop Imp. Assoc. 23rd Ann. Rept. **1941**: 32–38. 1942.
5. FAHMY, TEWFIK. The Fusarium disease (wilt) of cotton and its control. Phytopath. **17**: 749–767. 1927.
6. GILBERT, W. W. Cotton diseases and their control. U. S. Dept. Agr. Farmers' Bul. 1187. 1921.
7. HANSFORD, C. G. Cotton diseases of Uganda, 1926–1928. Empire Cotton Grow. Rev. **6**: 160–167. 1929.
8. ———. Cotton diseases of Uganda. Empire Cotton Grow. Rev. **6**: 240–245. 1929.
9. NEAL, D. C. Cotton diseases in Mississippi and their control. Mississippi Agr. Expt. Sta. Bul. 248. 1928.
10. RUDOLPH, B. A., and G. J. HARRISON. The unimportance of cotton seed in the dissemination of Verticillium wilt in California. Phytopath. **34**: 849–860. 1944.
11. TAUBENHAUS, J. J., and WALTER N. EZEKIEL. Seed transmission of cotton wilt. Science **76**: 61–62. 1932.

THE FIELD REACTIONS OF CERTAIN OATS HYBRIDS AND VARIETIES TO STEM RUST[1]

M. B. Moore, H. K. Hayes, and E. C. Stakman

(Accepted for publication March 12, 1945)

In the course of a breeding program for the production of disease-resistant varieties of oats, crosses were made in 1930–1933 between Bond and each of the varieties, Rainbow, Iogold, Anthony, "Double Cross" A,[2] and "Double Cross" B.[2] Selected lines from these crosses have been consistently resistant to stem rust *Puccinia graminis avenae* Erikss. and Henn. in the field until 1943. In this year, however, a liberal sprinkling of large pustules, in addition to the usual number of small ones, appeared on nearly all of the lines from Bond × Rainbow and Bond × Iogold crosses. These large pustules indicated the presence of races to which these varieties are susceptible. The lines from Bond × Anthony and from Bond × "Double

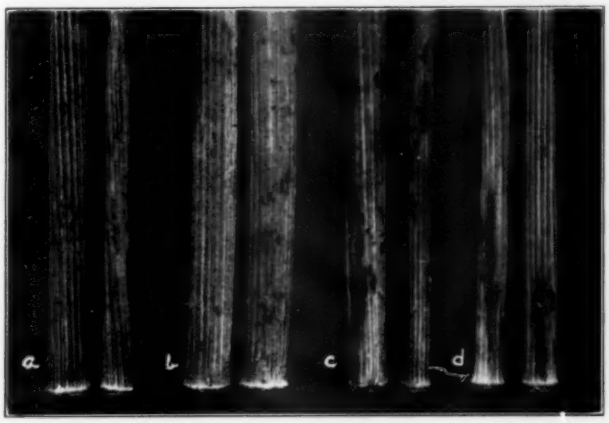


Fig. 1. Stem rust infection types on field grown plants. Semi-resistant type pustules on, a, Bond × "Double Cross"; b, Bond × Anthony. Semi-resistant and susceptible type pustules on c, Bond × Rainbow. Susceptible type pustules on d, Tama.

Cross" A and B, on the other hand, were resistant or semi-resistant as indicated by the development of the small pustules characteristically produced by races 2 and 5 on lines from all these crosses. On all of 26 lines of Anthony or "Double Cross" parentage there were only small pustules, and the lines were clearly semi-resistant or resistant. Of 25 lines of Rainbow or Iogold parentage, however, only 3 were completely semi-resistant or resistant, while both large and small pustules appeared on 22 lines (Table 1 and Fig. 1). Vicland and Tama likewise had both types of pustules, and in plots in various locations in Minnesota the prevalence was as high as 10 per cent, with the severity in some cases almost as high.

[1] Paper No. 2213 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

[2] Lines from (Minota × White Russian) × Black Mesdag.

TABLE 1.—*Classification of lines of oats for field reaction to stem rust at University Farm, St. Paul, Minn. in 1943*

Cross	No. of lines	No. of lines resistant or semi-resistant	No. of lines with resistance and susceptibility in same plant
Bond × Anthony	10	10	.....
" × Double Cross A	14	14	.....
" × Double Cross B	2	2	.....
" × Rainbow	15	3	12
" × Iogold	10	.....	10

The occurrence of the two types of pustules, often on the same plant, strongly suggested the presence of some race of stem rust other than the usual races 2 and 5. For this reason collections were made from each of the crosses and from Tama and Vicland and the rust was then identified in the greenhouse (Table 2). All of the 9 collections from lines or varieties having Rainbow, Iogold, or Richland parentage (Tama and Vicland from the last) yielded only race 8, while 3 of the 4 collections from the Anthony or "Double Cross" lines yielded only race 2 and one of them yielded 70 per cent of race 2 and 30 per cent of race 8. Such an occasional isolation of race 8 from these crosses, supposedly resistant to it, is to be expected since race 8 regularly causes small resistant type pustules on them.

It has recently been shown by Stakman and Loegering (3) that races 8 and 10, closely similar races, have increased during the last few years to 20.2 per cent of all of the collections made in the United States in 1943. In the same year races 2 and 5 comprised 79.1 per cent of the collections.

Presumably the resistance of the Anthony and "Double Cross" hybrids traces back to their White Russian parentage. Levine and Smith (1) found that White Russian and certain White Russian derivatives were resistant to races 1, 2, 5, 8, 9, and 10 and susceptible to 3, 4, 6, and 7 while Rainbow, Iogold, and Richland were resistant to 1, 2, 3, 5, and 7 and susceptible to

TABLE 2.—*Races of stem rust identified from lines and varieties of oats*[a]

Cross or variety	Number of lines from which rust races were identified	Races[b] and number of times identified	
		Races 2 & 5	*Races 8 & 10*
Bond × Anthony	2	2	
" × Double Cross A	1	1	
" × Double Cross B	1	1	1
" × Rainbow	5		5
" × Iogold	2		2
Tama	1		1
Vicland	1		1

[a] Inoculum taken from large susceptible type pustules whenever they were present.
[b] No distinction was made between races 2 and 5 or between races 8 and 10 since they differ only by a 4 or an X infection type on one differential variety.

4, 6, 8, 9, and 10. Smith (2) obtained evidence that resistance of the White Russian type to races 1, 2, and 5 was allelic to the resistance of Rainbow to 1, 2, 3, 5, and 7. He believed the same factor pair was responsible for reaction to races 8 and 9. As White Russian derivatives proved resistant in 1943 when race 8 was present, this is further indirect evidence regarding the White Russian factor for resistance. Welsh (4) in 1937 reported that lines obtained from a cross of Hajira and Joanette were resistant at low temperatures to races 1, 2, 3, 4, 5, 6, 7, 8, and 10. Nevertheless, resistance of seedlings to races 1, 4, 5, and 6 was influenced by temperature, and there was no surety that the resistance would be adequate under field conditions if high temperatures prevailed. It appears impossible to decide which of the three types of resistance to stem rust is most desirable, although the White Russian type was more satisfactory than the Rainbow-Iogold-Richland type in 1943.

The role of the barberry in permitting hybridization between races of *Puccinia graminis* and the resultant production of races having new pathogenic capabilities is well known. The possibility of virulent races acquiring, by the same means, factors for rapid multiplication, cold resistance, longevity—in short, for success in nature—should not be overlooked. Nearly all of the stem rust-resistant varieties of oats that have been grown extensively in northern United States have been of the White Russian or Rainbow-Iogold-Richland types as concerns reaction to stem rust. For a considerable number of years their resistance has been sufficient under practical field conditions to protect them against appreciable injury from stem rust. The field reactions of Richland, Rainbow and Iogold hybrids in 1943 show the desirability of further efforts to obtain oat varieties resistant to all prevalent races.

MINNESOTA AGRICULTURAL EXPERIMENT STATION,
ST. PAUL, MINNESOTA.

LITERATURE CITED

1. LEVINE, M. N., and D. C. SMITH. Comparative reaction of oat varieties in the seedling and maturing stages to physiologic races of *Puccinia graminis avenae*, and the distribution of these races in the United States. Jour. Agr. Res. [U.S.] **55**: 713–729. 1937.
2. SMITH, D. C. Correlated inheritance in oats of reaction to diseases and other characters. Minn. Agr. Exp. Sta. Tech. Bull. 102. 1934.
3. STAKMAN, E. C., and W. Q. LOEGERING. The potential importance of race 8 of *Puccinia graminis avenae* in the United States. Phytopath. **34**: 421–425. 1944.
4. WELSH, J. N. The synthetic production of oat varieties resistant to race 6 and certain other physiologic races of oat stem rust. Can. Jour. Res. (C) **15**: 58–69. 1937.

NIGROSPORA ORYZAE (B. AND BR.) PETCH ON MAIZE[1]

J. H. STANDEN[2]

(Accepted for publication, March 17, 1945)

INTRODUCTION AND REVIEW OF LITERATURE

A rot caused by *Coniosporium gecevi* B. and K. in ears of maize in Bulgaria was described in 1912 by Bubak and Kosaroff (3). Durrell (6) in 1925 found that the same fungus, which he referred to *Basisporium gallarum* Moll., invaded maize kernels and injured the embryo. He observed that infection was more prevalent in early varieties than in late ones, and that the disease was severe following heavy rains in August and September. Savulescu and Rayss (19 through 25) found that Nigrospora infection of maize in Roumania was prevalent in varieties with a high water content in the ears at time of harvest. According to their observations, late varieties were more frequently infected than early varieties. This was an apparent contradiction of Durrell's observations. According to Raleigh (17) Nigrospora-infected seed often produced plants with normal ears if there was no competition in the hill, but in competition with strong, healthy plants, plants from such infected seed seldom produced normal ears.

Reddy (18) reported that infection by *Nigrospora* occurred after translocation ceased in the plant. He found that Nigrospora-infected seed suffered the most damage at temperatures too low for rapid germination. Seeds of certain inbred lines of maize germinated at lower temperatures than others and suffered less damage from infection.

Seed treatment was most beneficial under conditions unfavorable to germination of the seed. Reddy found that incidence of infection was lower in cobs with a low pH than in those with a high pH.

In an effort to extend and clarify our knowledge of this disease a study was made on the pH of the cob, on the maturity of the host in relation to prevalence of infection, and on the overwintering of the fungus.

DESTRUCTIVENESS, GEOGRAPHICAL DISTRIBUTION, AND PREVALENCE OF THE DISEASE

Infection of maize plants by *Nigrospora* has been reported so widely that the disease probably exists wherever maize is grown. The fungus, under several different names, has been reported on maize in North America, Asia, Europe, New South Wales, and Java (Table 1). In 1939 the author collected it on an ear of ceremonial corn obtained from the Indian Day School, Taos, New Mexico.

[1] Journal paper No. J–1234 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 95. This paper constitutes one section of a thesis submitted to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The author wishes to express his gratitude to Drs. C. S. Reddy and I. E. Melhus for their guidance in this research, and to the many others who have given valuable assistance.

Although the disease is widespread, most of the estimates of losses to the corn crop have been made for the corn belt of the United States. The loss in Iowa for 1923 to 1939 was estimated at 3 per cent (Iowa Plant Disease Survey records of the Botany Department, Iowa State College). In 1923 a loss of 30 per cent was recorded for certain cornfields in the State, while in 1925 the average loss for the State was 8 per cent. From 1924 to 1938 Nigrospora dry rot caused a loss in two or more years of 0.5 per cent or more in Illinois, Wisconsin, Indiana, and Iowa, according to reports published in Plant Disease Reporter Supplements. Losses were more serious in some years than in others. The disease was also reported during this period from

TABLE 1.—*Geographical distribution of Nigrospora oryzae on maize and synonyms under which the fungus has been reported*

Locality	Fungus	Date
North America		
Ohio	*Coniosporium gecevi* Bubak	1912 (1)
Iowa	*Basisporium gallarum* Moll.	1925 (6)
Manitoba, Canada	*Basisporium gallarum*	1938 (2)
New Mexico	*Nigrospora oryzae*	1939
Europe		
Italy	*Trichosporium maydis* Sacc.	1911 (12)
Bulgaria	*Coniosporium gecevi* Bubak	1912 (3)
Roumania	*N. oryzae* (B. and Br.) Petch	1930 (20)
North Caucasia	*N. oryzae*	1933 (11)
Asia		
Java	*Nigrospora javania* Zimm.	1918 (16)
Western Siberia	*Basisporium gallarum*	1935 (14)
Africa		
Gold Coast, Africa	*B. gallarum*	1926 (4)
Kenya Colony, Africa	*N. sphaerica* (Sacc.) Mason	1929 (13)
Tanganyika Territory, Africa	*N. sphaerica*	1931 (28)
Australasia		
New South Wales	*B. gallarum*	1931 (15)

Connecticut, Ohio, Kansas, Missouri, Minnesota, and Massachusetts. Hoppe and Holbert (8) isolated *Nigrospora* frequently from damaged kernels from corn belt states, but from only a small percentage of the kernels from southern and western United States. Koehler and Holbert (10) estimated an average annual loss in Illinois of a little over 1 per cent, which may not be too high for the corn belt states as a whole.

Savulescu and Rayss (20) have reported the disease in Roumania, where the fungus caused important losses in dent corn.

The fungus invaded the maize shank, the cob, and the kernels. Durrell (6) found that shanks of infected ears were at times so severely rotted that the ears were knocked to the ground by mechanical pickers. The invasion of the seed, however, probably is responsible for the larger part of the loss caused by this fungus.

Durrell (6) showed that the viability of infected seed was considerably lower than that of nearly disease-free seed. Koehler, Dungan, and Holbert (9) in 1928 found that, on good soil, seed infected with *Nigrospora* yielded 59 bushels as compared with 65 bushels for nearly disease-free seed.

Many of the inbreds and single crosses employed in producing hybrid seed corn were very susceptible to *Nigrospora*. In 1938 infection was found in 11 of 42 ears of inbred L–317, 9 of 30 ears of Os–426, and 28 of 38 ears of I–205. In the same year corn in a 40-acre crossing block in eastern Iowa was so severely infected as to be unfit for seed.

Seed corn may appear free of disease and still be severely infected with *Nigrospora*, as indicated by the following experiment. Two rows of kernels were taken from each of 20 ears known to be infected. After removal of kernels that appeared to be damaged, samples were submitted to each of three Federal grain grading agencies, by which they were given a uniform grade of No. 1 sound corn. Kernels were then surface disinfected and dropped on agar slants. *Nigrospora* was on 334 of 408 kernels, ***Fusarium***

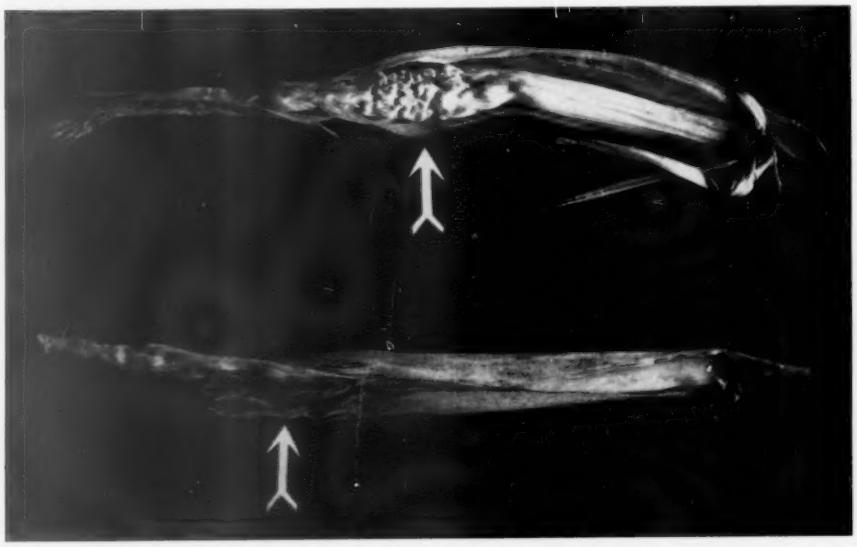


FIG. 1. Arrested axillary shoots of corn infected by *Nigrospora*. Arrows point to areas severely retted by the fungus.

or other fungi occurred on 58, while 16 appeared to be disease-free. Infected kernels failed to germinate or germinated less vigorously in the agar slants than healthy kernels.

Almost all references to *Nigrospora* in the literature are concerned with the dry rot of the ear. Ellis[3] collected infected maize culms in New Jersey, and Durrell (5) reported nodal infection. The author has published a note (26) on the prevalence of this fungus in arrested axillary shoots and poorly-developed secondary ears. In some collections, each of 40 or more arrested axillary shoots were infected. Collections with 50 to 80 per cent of the shoots infected were general. Infection was far more common in small, poorly-developed secondary ears than in ears that were well developed. Two Nigrospora-infected arrested axillary shoots are shown in figure 1. The *Nigro-*

[3] No. 968, North American Fungi, J. B. Ellis.

spora in these tissues, while of little economic importance in itself, probably provided an abundance of field inoculum. Spores from infected axillary shoots overwintering in the field were viable in October of the following year.

OVERWINTERING AND GERMINATION OF NIGROSPORA SPORES

An experiment was arranged to gain some idea of the rôle of the abundant inoculum produced in arrested axillary shoots and poorly-developed secondary ears in the initiation of infection the following season. In the fall of 1938 one lot of infected maize tissues, including ears and arrested axillary shoots, was buried 2 inches in the soil. A second lot was placed on the surface of the soil and covered with a screen wire frame to prevent dispersal by the wind. At intervals in the following spring spores from the two lots and from stalks standing in the field were tested for germination by the method of Durrell (6). The spores were scattered on a microscope slide

TABLE 2.—*Viability of Nigrospora spores from infected maize tissues buried in the soil, stored on the surface of the soil, and left on standing stalks over winter*

Source of spores and date on which overwintering began	Percentage germination of spores in the following spring and summer			
	Mar. 30	June 2	Aug. 10	Oct. 3
Fall, 1938				
Buried tissues	0	.....	.....	.....
Tissues on soil surface	15–20	15	15–20	10
Standing stalks	50–60	50–60	.....	.....
	Mar. 26	June 5		Oct. 1
Fall, 1939				
Tissues on soil surface	20	15–20	.....	10
Standing stalks	70	60–65	.....	.....

with a little water and the slide placed in a Petri dish with a few green leaves for 12 to 18 hours. Data from these tests and from tests made on material stored for overwintering in the fall of 1939 are in table 2. The spores from infected tissues overwintering on standing stalks germinated much better than spores from tissues on the surface of the soil, while spores from buried tissues failed to germinate. The experiments with buried tissues were not repeated in 1939–1940. By early June most of the standing stalks with arrested axillary shoots had been plowed down or the stalks had been stripped by the wind. Ten per cent of the spores from tissues on the surface of the soil germinated long after infection had occurred in the field in each of the two years. Spores from material placed on the soil surface in 1938 were viable on Oct. 3, 1939, while similar material stored in the fall of 1939 was still viable on Oct. 1, 1940.

A few spores in tissues stored in the laboratory were viable after five years: spores from a piece of infected cob stored in the laboratory in 1934 were placed in a germinator in 1939, and of 500 or more spores, 2 germinated.

Many of the spores from the tissues that were buried in the ground over

winter were broken in a circumcissal manner which gave them a pill-box appearance. The fracture was perfectly smooth and always in the same plane, namely, a little to one side and parallel to the equatorial plane of the oblate spheroidal spores. Durrell (6) noted a line of fracture extending half way around spores that he had treated with nitric acid.

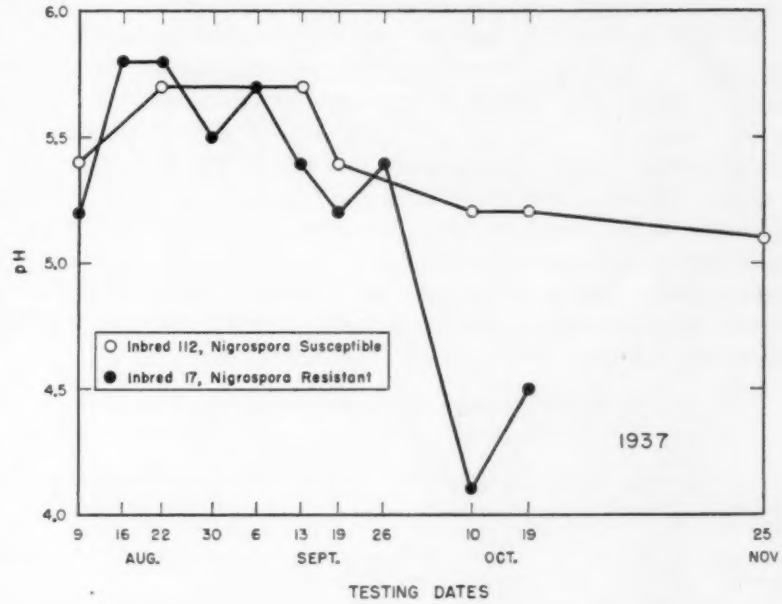
SLIGHT NIGROSPORA INFECTIONS IN EARS OF MAIZE

Cobs so slightly infected with *Nigrospora* as to escape detection on microscopic examination were of frequent occurrence. Such infections were detected by soaking the ears in water about 12 hours, incubating at 100 per cent humidity at room temperature for 4 days, and slowly drying for about a week. Shorter periods of incubation did not allow all of the slight infections to become evident. Examination without the drying period likewise did not permit the detection of all of the slight infections. Using this technique, well-matured maize ears were tested from eight Iowa localities in 1937 and 1938. Of 365 ears subjected to microscopic inspection without the treatment, 10 were found infected. Of 365 ears subjected to the soaking, incubation, and drying treatment, infection was detected in 66. This would indicate the possibility that slight infections in the field in early autumn may become extensive if the corn is allowed to remain in the field under conditions favorable to the fungus. Such a possibility is of especial importance in the relation to seed corn.

RELATION OF pH OF COBS TO INCIDENCE OF INFECTION

Reddy (18) observed that corn inbreds in which the pH of the cobs was high were frequently infected with *Nigrospora,* while infection was less common in inbreds in which the cob pH was low. He examined 410 ears in 1932 in which the pH ranged from 4.40 to 4.59 and found one infected ear. Infection was found in 64 of 248 ears in which the pH ranged from 5.40 to 5.79. In experiments with the effect of pH on growth of the fungus in pure culture no differences in response of *Nigrospora* were observed over a range of pH exceeding that occurring in the host plant.

In an attempt to explain the relation of cob pH to infection in the field, successive pH readings were made on cobs of corn inbreds with high or low incidence of *Nigrospora* during 1937 and 1938. The data on these tests are recorded graphically in figure 2. In the early part of the season pH determinations were on the entire immature ears. Kernels in the milk stage were scraped from the cobs before testing. Later tests were on the cobs with kernels removed. Although the pH was uniform for all inbreds early in the season, there was a drop in cob pH of inbreds with a low incidence of infection as the ears became mature. In 1938 three of six cobs in the low incidence groups harvested October 3, had a pH of 5.2, 5.5, and 5.6, respectively. All three ears were poorly matured, and notations before testing were that the pH should be high. The evidence obtained in these experiments indicated that an ear that matured well had a cob with low pH. This fact,



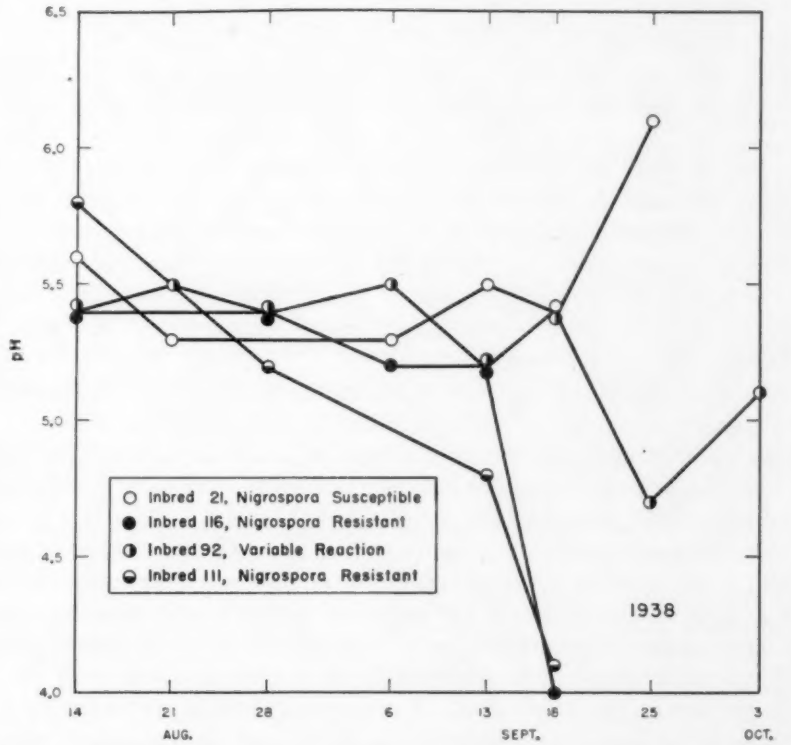


FIG. 2. Seasonal variation in cob pH of Nigrospora-resistant and Nigrospora-susceptible inbreds. (Each point is average pH reading of 3 ears.)

coupled with Reddy's observations and with earlier work by Standen (27), indicates that incidence of infection was probably dependent on maturity.

Inbreds 92[4] and 21 presented contrasting examples of the relation of maturity to infection. Inbred 21 died early in the field in 1937 and 1938. Inbred 92 was late in maturing in both years. In 1937 three frosts had occurred by the middle of September, and inbred 92 was killed while still immature. Infection was found in 14 of 47 ears in that year. In 1938 the first frost occurred late in the fall at Ames, and only 2 ears of 104 harvested from inbred 92 were infected. Inbred 21 had Nigrospora infection in 15 of 59 ears in 1938. This would indicate that, under certain conditions, inbreds dying prematurely and inbreds that are still immature at time of frost may be attacked by *Nigrospora*.

THE RELATION OF MATURITY AND DEATH OF THE HOST TO INCIDENCE OF INFECTION

Infection was abundant in arrested shoots and poorly-developed secondary ears. In 1938, infection was observed in 50 of 100 ears harvested from stalks so severely lodged that the ears rested on the ground. In the same field 13 of 603 ears harvested from standing stalks were infected. If the difference in moisture relationship did not entirely account for the difference in infection, this might indicate a relationship between poor maturity[5] and high incidence of infection. Experiments were arranged to obtain more evidence on this relationship.

TABLE 3.—*Relation of maturity of maize plants on September 11, and incidence of Nigrospora infection in the ears on November 19, 1938*

Maturity of plants	No. single crosses	No. ears examined	Ears infected	
			No.	Per cent
Well matured	101	4923	243	4.9
Prematurely dying	69	2717	731	19.7
Immature	66	3010	206	6.8

A series of 236 single crosses was planted in 1938 to provide material with a wide range of maturity. On September 11 the plants were examined and on the basis of apparent maturity classified into three groups, as prematurely dying or dead, immature, and well matured. The ears were harvested November 19 and examined for infection (Table 3).

The greatest amount of infection was in the group classified as prematurely dying, in which 19.7 per cent of the ears were infected; but the infection (6.8 per cent) in ears of the immature single crosses was significantly greater than the 4.9 per cent in ears from the well-matured crosses.

The fungus grew more abundantly on poorly-matured than on well-matured ears in the laboratory. Ears were soaked in water for 12 hours

[4] The inbred lines of maize designated by number only are from a series carried by C. S. Reddy of the Iowa Agricultural Experiment Station.

[5] The word "immature" designates living tissues that have not reached maturity. Poorly matured tissues are those that have died without becoming matured.

TABLE 4.—*Inoculation of maize ears with Nigrospora*

Corn sample and year tested	Checks				Treated ears	
	Not inoculated, not incubated		Not inoculated, incubated		Inoculated and incubated	
	Examined	Infected	Examined	Infected	Examined	Infected
1938						
Commercial corn	.....	.....	19	4	42	35
1939						
Commercial corn	35	0	12	0	35	35
Commercial corn	18	0	.....	.....	18	18
Inbreds'	29	3	30	12	30	28
Total	82	3	61	16	125	116

and a portion of Nigrospora culture applied to the butt of the ear. The ears were then incubated and dried as for detection of slight infection. Data from four tests which included untreated checks and ears incubated but not inoculated (Table 4) show that a high percentage of ears that were inoculated and incubated became infected. The commerical corn samples were selected, well-matured ears. In none of these infected ears was sporulation detected at a depth in the cob of more than $\frac{3}{4}$ inch. The inbred ears were poorly matured. Sporulation occurred at a depth exceeding $1\frac{1}{2}$ inches in 22 of the 28 infected inbred ears. In the six ears in which infection was not so extensive, other fungi such as *Monotospora* or *Fusarium* were established as well as the *Nigrospora*. The limited growth of *Nigrospora* may have been due to these other organisms.

The retting of the cob, a characteristic symptom of the disease in the field, was produced in the laboratory. Twelve samples of poorly-matured cob were autoclaved, with sufficient water to keep them moist, in half-pint bottles and inoculated with *Nigrospora* in pure culture. Each sample was completely retted in two or three weeks. Twelve well-matured samples subjected to the same treatment were not retted. Growth of the fungus was more vigorous on the poorly-matured than on the well-matured cobs. To twelve samples of well-matured cobs was added extract of poorly-matured cob. Growth of the fungus was abundant on these samples, but the cobs were not retted.

In the retting experiment tests were made for pectinase, using the method

TABLE 5.—*Relation of condition of arrested axillary shoot tissues on September 11, 17, and 24, to initiation of Nigrospora infection*

Condition of tissues	Infection by *Nigrospora*					
	September 11		September 17		September 24	
	Examined	Infected	Examined	Infected	Examined	Infected
Green	3	0	6	0	23	0
Partly green, discolored	19	4	4	0	.....	.....
Soft and discolored	26	14	17	13	19	18

of Harter and Weimar (7) in which the enzyme is detected by its ability to soften the tissues of sweet potato. No pectinase was found in six separate tests with extracts of cobs retted in the laboratory, but in each of four tests with naturally retted maize tissues the pectinase was present.

A study of arrested axillary shoots in 1938 furnished evidence on the relation of maturity and death of the host tissues to incidence of infection. Infection was very common in these shoots. Shoots from inbred lines of corn were examined for evidence of infection on September 11, 17, and 24 (Table 5).

In all collections in which infection could not be detected by the presence of spores, portions of the tissue were cultured. In no case was *Nigrospora* detected in portions of the shoots that were still green and firm. In the collections made September 24, the 19 soft and discolored shoots, of which 18 were infected, were gathered from consecutive plants of an inbred in which the shoots became soft and disintegrated early in the fall, while the 23 green, firm shoots were taken from consecutive plants of two inbreds growing a few feet from the plants bearing infected shoots. All shoots were rather uniformly exposed to infection, but infection occurred only in tissues that were becoming soft and discolored.

Evidence on the relation of premature death of plants in the field to prevalence of ear infection was obtained in the fall of 1940. Several inbreds died prematurely after heavy rains in late summer. One of these, inbred 113, is shown in figure 3 beside a row of inbred 116, the ears of which were well matured. Infection was found in 99 of 123 ears of inbred 113, and in only one of 126 ears of inbred 116. Inbreds 102, 103, and 104 also died prematurely. Infection was found in 14 of 17, 8 of 24, and 5 of 27 ears of these lines.

Because poorly-matured plants were most severely attacked by *Nigrospora* and because poor maturity is frequently related to the condition of the plant roots, an experiment was arranged to test the condition of the roots as indicated by the force required to pull the plant from the ground. Two groups of inbreds were tested, one group in which none of the ears was infected in 1938, and the other in which from 10 to 50 per cent of the ears were infected. Consecutive 3-plant hills of each inbred were pulled by means of a lever and spring scale, and the required force recorded in pounds. In 1938 and 1939 the average force required to pull inbreds in which the incidence of infection was low was almost twice that required for inbreds severely attacked by *Nigrospora* (Tables 6 and 7).

The force necessary to pull a corn plant has a limited bearing on the condition of the ear or of the plant as a whole. The roots may be in good condition, but naturally shallow and therefore relatively easy to pull. This was true of the three inbreds, 90, 74, and 107, in 1938. On the other hand the roots may be in good condition but the stalk severely rotted. This was true in 1939 of inbred L–317, the ears of which were poorly matured. The difference in average pulling force required for the two groups of inbreds, how-

ever, was sufficient to indicate strongly a relationship between root condition and incidence of infection by *Nigrospora*. No attempts were made during the investigation to determine the cause underlying the weakening of the

FIG. 3. Condition of two inbreds, the one with severe Nigrospora infection in ears, the other with little Nigrospora infection following heavy fall rains in 1940. Left, Inbred 116; Nigrospora infection in 1 of 126 ears. Right, Inbred 113; Nigrospora infection in 99 of 123 ears.

maize roots. It was considered possible that inbreds might have a different response, insofar as root condition was concerned, to different seasons and

TABLE 6.—*The force required to pull maize plants with high or low incidence of Nigrospora infection in ears, November 20, 1938*

High incidence of *Nigrospora*			Low incidence of *Nigrospora*		
Inbred line No.	Force in lb. to pull 1 hill		Inbred line No.	Force in lb. to pull 1 hill	
	Range[a]	Av.		Range[a]	Av.
99	10–164	95.4	105	146–260	221.8
L–317	52–144	101.2	115	88–270	180.0
102	52– 96	70.8	116	68–270	164.2
2	5–128	73.1	91	80–164	113.0
113	7– 60	32.0	52	84–236	158.4
L–287	100–260	147.0	90	36–108	72.8
80	14–164	95.4	30	64–232	151.6
21	12– 96	55.0	101	88–192	138.4
81	28– 92	56.4	74	36–184	98.0
85	10– 80	34.9	107	20–128	87.6
	Average of all hills	76.1		Average of all hills	138.4

[a] Based on 10 individual hills.

soils. In the light of the evidence presented on the relation of incidence of infection to poor maturity and premature death of the host, the observations of other investigators are of interest.

Hoppe and Holbert (8) observed that *Nigrospora* was important as an ear rot fungus in those years when the maturity of the corn crop was affected by early frost. Koehler and Holbert (10) in describing the symptoms of Nigrospora dry rot, said that severely-infected ears frequently were chaffy. The evidence presented in the present paper indicated that chaffy ears were attacked because of their poor maturity rather than that the chaffiness was caused by *Nigrospora*.

TABLE 7.—*The force required to pull maize plants with high or low incidence of Nigrospora infection in ears, October 20, 1939*

High incidence of *Nigrospora*			Low incidence of *Nigrospora*		
Inbred line No.	Force in lb. to pull 1 hill		Inbred line No.	Force in lb. to pull 1 hill	
	Range[a]	Av.		Range[a]	Av.
La–317	30–200	106.0	115	200–365	285.5
102	22–126	67.6	116	155–500	336.5
113	58– 98	77.6	91	102–210	163.7
2	62–130	82.8	52	50–360	163.5
L–289	65–260	178.9	90	65–138	107.7
80	30–102	61.4	30	130–340	252.3
81	4–178	68.3	101	22–238	148.8
85	14–238	107.8	74	150–350	210.0
L–289B	115–170	143.0	107	65–210	101.5
I–205	50–240	132.0	14	158–270	212.5
	Average of all hills	103.5		Average of all hills	191.6

[a] Based on 10 individual hills.

According to Ryker[6] *Nigrospora* "is not responsible for any disease in rice, although a constant companion of the plant," and he has "never found any indication of its being parasitic. It occurs on almost all growing rice and if for any reason tissue is injured, it develops abundantly in that area. For this reason, it is very bothersome in that in plating of diseased tissue it masks the causal organism."

SUMMARY AND CONCLUSIONS

Poorly-matured maize tissues were more susceptible to infection by *Nigrospora* than well-matured tissues. Single crosses, separated on the basis of visual inspection into poorly-matured and well-matured groups, had a far greater incidence of infection in the poorly-matured than the well-matured group. Such factors as lodging, stalk rot, root rot, and early frosts, all contributed to increased incidence of Nigrospora infection in ears of maize. Secondary ears and arrested axillary shoots, because of their poor maturity, had a high incidence of infection.

The relation between the pH of the cob and incidence of infection, previously reported by Reddy (18), depended on the lowering of the cob pH as the ear matured. In cobs that did not mature well, or that were not mature at the time of frost, the pH remained high. Late-maturing inbreds were heavily infected in years of early frost, and free of infection when frost was delayed. Green tissues did not become infected.

Infection had been initiated in many maize ears in which *Nigrospora* failed to spread. The growth of the fungus following infection appeared to be conditioned by moisture, temperature, and the maturity of the cob. The retting of cob tissues, characteristic of the disease in the field, was accomplished in the laboratory, but only by use of poorly-matured cobs. Chaffiness of the ear has been described as a characteristic of the disease in the field. It is probable that chaffy ears are infected because they are poorly matured.

Arrested axillary shoots and poorly-developed secondary ears had a high incidence of Nigrospora infection. It is probable that these tissues constitute a principal source of inoculum for the following season.

IOWA AGRICULTURAL EXPERIMENT STATION,
AMES, IOWA.

LITERATURE CITED

1. ARZBERGER, E. G. The cob-rot of corn. Ohio Agr. Exp. Stat. Bull. 265: 69–82. 1913.
2. BISBY, G. R., and I. L. CONNORS. Plant diseases new to Manitoba. Scientific Agr. **8**: 456–458. 1928.
3. BUBAK, FR., und P. KOSAROFF. Einige interessante Pflanzenkrankheiten aus Bulgarien. Centralbl. Bakt., II Abt., **31**: 500–501. 1912.
4. BUNTING, R. H. Annual report. Gold Coast Dept. Agr. Annual Report 1925/1926: 32–33. 1926. (Original not seen. Abstr. in Rev. App. Myc. **6**: 144. 1927.)
5. DURRELL, L. W. A preliminary study of fungous action as the cause of down corn. Phytopath. **15**: 146–154. 1925.
6. ———. Basisporium dry rot of corn. Iowa Agr. Exp. Stat. Res. Bull. 84: 139–160. 1925.

[6] T. C. Ryker, University of Louisiana, in correspondence.

7. HARTER, L. L., and J. L. WEIMAR. Studies in the physiology of parasitism with special reference to the secretion of pectinase by *Rhizopus tritici*. Jour. Agr. Res. [U.S.] **21**: 609–625. 1921.
8. HOPPE, P. E., and J. R. HOLBERT. Relative prevalence of various ear rot fungi in the 1933, 1934, and 1935 corn crops. U. S. Dept. Agr., Plant. Dis. Reptr. **20**: 312–320. 1936.
9. KOEHLER, B., G. H. DUNGAN, and J. R. HOLBERT. Plant disease investigations at the Illinois Station. Ill. Agr. Exp. Stat. Ann. Rpt. No. 41: 69–73. 1928.
10. KOEHLER, B., and J. R. HOLBERT. Corn diseases in Illinois. Ill. Agr. Exp. Stat. Bull. 354. 1930.
11. LOBIK, A. I. [Present position of the problem of the diseases and injuries affecting maize in North Caucasus.] Bull. N. Caucasian Inst. for Plant Protection, Rostoff-on-Don 1(8), part 2: 3–51. 1933. (Abstr. in Rev. App. Myc. **12**: 505. 1933.)
12. MASON, E. W. On species of the genus *Nigrospora* Zimmermann recorded on monocotyledons. Trans. Brit. Mycol. Soc. **12**: 152–165. 1927.
13. MCDONALD, J. Annual report of the mycologist. Kenya Colony, Dept. of Agr., Ann. Rept. 1929: 464–479. 1930.
14. MOURASHKINSKY, K. E. [New diseases of cultivated plants in Western Siberia.] Trans. Omst. Inst. Agr. **6**: 3–30. 1935. (Abstr. in Rev. App. Myc. **14**: 493. 1935.)
15. NOBLE, R. J. New South Wales: plant diseases. Internat. Bull. of Plant Prot. **5**: 202–205. 1931.
16. PALM, B. Einige ziekten, waargenomen an de tarwe op Java. Batavia, Meded. Labor. v. Plantenz. No. 34, 22 p. (De *Nigrospora* ziete. p. 17–18.) 1918.
17. RALEIGH, W. P. Infection studies of *Diplodia zeae* (Schw.) Lev. and control of seedling blights of corn. Iowa Agr. Exp. Stat. Res. Bull. 124: 96–121. 1930.
18. REDDY, C. S. Resistance of dent corn to *Basisporium gallarum* Moll. Iowa Agr. Exp. Stat. Res. Bull. 167. 1933.
19. SAVULESCU, T. L'état phytosanitaire en Roumanie au cours de l'année 1928–1929. Ann. Inst. Recherches Agron. de Roumanie **1**: 214–266. 1930.
20. SAVULESCU, T., and T. RAYSS. Une nouvelle maladie du mais en Roumiane provoquée par *Nigrospora oryzae* (B. et Br.) Petch. Arch. Roumanies Path. Exp. et Microbiol. **3**: 41–53. 1930.
21. ———— and ————. Contribution a la connaissance de la biologie de *Nigrospora oryzae* (B. et Br.) Petch, parasite du mais. ex Trav. Cryptog. dédiés a Louis Mangin, p. 233–240. Museum National d'Hist. Nat., Paris. 1931.
22. ———— and ————. Influence des conditions extérieures sur le développement de *Nigrospora oryzae* (B. et Br.) Petch, parasite du mais en Roumanie. Acad. Sci. (Paris) Compt. Rend. **194**: 1262–1265. 1932.
23. ———— and ————. Der Einfluss der ausseren Bedingungen auf die Entwicklung der *Nigrospora oryza* (B. und Br.) Petch. Phytopath. Zeitschr. **5**: 153–172. 1932.
24. ———— and ————. [Dry rot of maize cobs in Roumania.] Ann. Inst. Recherches Agron. de Roumanie **5**: 3–112. 1933. (Abstr. in Rev. App. Myc. **14**: 493. 1934.)
25. ———— and ————. La resistance de differentes lignies de blé au *Nigrospora oryzae* (B. et Br.) Petch et une methode nouvelle pour la determination de l'effect parasitaire. Int. Bot. Cong. Proc. **2**: 208–210. 1935.
26. STANDEN, J. H. Prevalence of *Basisporium gallarum* in arrested axillary shoots and secondary ears of maize. Phytopath. **29**: 656–657. 1939.
27. ————. Chemical and physical characteristics of maize cobs in relation to the growth of *Nigrospora oryzae*. Phytopath. **34**: 315–323. 1944.
28. WALLACE, G. B. Report of the mycologist. Tanganyika Territory, Dept. Agr. Ann. Rpt. for 1930. pp. 14–97. 1932.

ANTHRACNOSE OF GARDEN PEA

S. H. Ou[1] and J. C. Walker

(Accepted for publication March 17, 1945)

INTRODUCTION

Anthracnose (*Colletotrichum pisi* Pat.) of pea (*Pisum sativum* L.) was first collected in Ecuador in 1891 (11). Since then it has been noted in Wisconsin (7, 8, 17), Connecticut (4), Louisiana (12), Canada (2, 5), Japan (6), and Russia (1). The surveys of pea diseases in Wisconsin made by Jones and Linford (7) and Walker and Hare (17) revealed that the disease was very prevalent in both 1924 and 1942.

The disease as it appears in the field has been described by Jones and Linford (7) and illustrated by Walker and Hare (17). Brown lesions occur on leaves, stems, and pods. It was pointed out by Walker and Hare (17) that the symptoms are not readily distinguished in their early stages from those of *Ascochyta spp.* and that the two diseases often occur on the same plant. Ordinarily the development of setae and sclerotia of *Colletotrichum pisi* in the lesions has been taken as the basis of distinction of anthracnose from Ascochyta blights. It will be shown later in this paper, however, that *Ascochyta spp.* and *C. pisi* occur very commonly together in the same lesion and that on stems, particularly, the latter does not infect independently. It should be pointed out, therefore, that the symptoms which have been ascribed ordinarily to anthracnose are, in fact, those of the combined effects of one or another species of *Ascochyta* and *C. pisi.*

The present paper is concerned with isolation and inoculation experiments, the interaction of the causal fungus with *Mycosphaerella pinodes* (Berk. & Blox.) Stone, the *Ascochyta* stage of which is most commonly associated with *Colletotrichum pisi* in Wisconsin, and the transmission of the latter organism with pea seed.

ISOLATION EXPERIMENTS

Colletotrichum pisi grows readily on common artificial media. Cultures were obtained usually by placing fragments of diseased tissue in 1–1000 mercuric chloride for one minute and plating on potato-dextrose agar. In 1942, many of the resulting colonies contained both *C. pisi* and *Ascochyta spp.* Beginning early in the season of 1943, isolations were made at intervals of 7 to 10 days from diseased plants collected in 5 localities in central Wisconsin. Fifty or more lesions were used from each collection. At the time of the first isolations no acervuli or sclerotia were present on the brown lesions. About 75 per cent of the resulting colonies contained *Ascochyta spp.* as well as *C. pisi.* Ten days later lesions had appeared on the stems, and setae of the anthracnose organism were present. Young stem lesions bearing

[1] Acknowledgment is made to the China Foundation for the Promotion of Education and Culture for its encouragement to and financial support of the senior author during the period of his graduate studies at the University of Wisconsin.

setae, selected under a binocular, were removed from the stem and plated. Of about 300 colonies, nearly 100 per cent contained both *C. pisi* and *Ascochyta spp.* In later isolation experiments, when selerotia had appeared on the stem lesions, the percentage of colonies containing *Ascoschyta spp.* gradually decreased. Thus, it became evident that *C. pisi* was commonly associated with *Ascochyta* in the field.

INOCULATIONS

Jones and Vaughan (8) obtained positive results on leaves and stems inoculated in the field. Antonova (1) stated that infection of pea seedlings was secured with difficulty and only in the presence of wounds.

The organism, as isolated from naturally infected plants, produced no spores on potato-dextrose agar, but it produced abundant, atypical, cylindrical conidia in a number of liquid media. Conidia produced in modified Duggar's solution[2] were usually used for inoculation. A strain which produced normal falcate conidia on potato-dextrose agar was later isolated from a sector of the original mycelial culture. This strain also produced cylindrical conidia in liquid media. The pathogenicities of the mycelial and conidial types were compared and found to be similar.

In this study, a large number of inoculations was made in the greenhouse, usually on Perfection or Prince of Wales varieties. At least 3 pots, each containing 10 to 12 pea plants, were used for each treatment. They were inoculated when the fifth or sixth leaf had expanded and the plants were 8 to 10 inches high. Beyond this stage the first or second leaf tended to show signs of senescence, and it was difficult, therefore, to determine whether infection had occurred on living or dead tissue. The pots were placed in a glass moist chamber for 12 hours before and 24 hours after inoculation. Spore suspensions were applied by means of an atomizer. The moisture in the chamber was controlled by an atomizer which was so adjusted that nearly 100 per cent relative humidity was kept without an excess of free water accumulating on the plant. The temperature in the chamber varied from 20° to 23° C.

The results of a number of similar inoculations varied somewhat, but as a rule an average of 3 to 4 leaflets of each plant, usually the lower ones, became infected. The lesions appeared as small, depressed, water-soaked areas within 2 days after inoculation. Unlike the field symptoms, no brown color was evident until the leaflets were dead. Reisolation from these infected leaflets usually yielded the organism.

When inoculation and incubation were carried out at 16°, 20°, 24°, and 28° C., the number of infected lower leaflets increased with temperature. This thermal response was undoubtedly due in part to the influence of temperature upon the growth of *Colletotrichum pisi.* It was also evident, how-

[2] Composition of the solution: M/2 dextrose, 500 ml.; M/2 KNO_3, 200 ml.; M/4 KH_2PO_4, 200 ml.; M/10 $MgSO_4 \cdot 7H_2O$, 100 ml.; M/100 Fe-tartrate, 1 ml.; A–Z solution, 1 ml. The A–Z solution consisted of: LiCl, 0.5 g.; $CuSO_4 \cdot 5H_2O$, 1.0 g.; $ZnSO_4$, 1.0 g.; H_3BO_3, 11.0 g.; $Al_2(SO_4)_3$, 1.0 g.; $SnCl_2 \cdot 2H_2O$, 0.5 g.; $MnCl_2 \cdot 4H_2O$, 7.0 g.; $NiSO_4 \cdot 6H_2O$, 1.0 g.; $Co(NO_3)_2 \cdot 6H_2O$, 1.0 g.; TiO_2, 1.0 g.; KI, 0.5 g.; KBr, 0.5 g.; H_2O, 18 liters.

ever, that at 24° and 28° C. the lower leaves of pea plants became senescent more rapidly, and this was probably an equally important factor in enhancing disease development.

In no case in these and many other experiments were any stem lesions noted up to one month after inoculation. Microscopic examination of inoculated stems and leaflets without lesions showed numerous germinated conidia and many appressoria. In a few cases the inoculated plants were kept in the moist chamber for a longer period. Small colonies of the organism developed on the surface of the stem, but no lesions resulted. The control plants showed no disease except an occasional wilting leaflet which yielded no fungus when plated.

Apparently healthy pods were picked from the field at about the canning stage, washed in sterile water, and inoculated. The pods were kept in moist chambers in an incubator at 20° C. Water-soaked, depressed lesions were evident in 5 or 6 days. The lesions reached 4 to 5 mm. in diameter in about 10 days and usually small, superficial sclerotia appeared at the center. The control showed no disease development. Four lots of pods of 4 varieties were tested with similar results. Examination of a large number of these pods showed that the fungus seldom penetrated through the inner pod wall, and no infection of the seed underneath was macroscopically evident after 3 weeks. Seeds from pods of the same stage were removed aseptically into a small sterilized moist chamber and were inoculated with conidia. Lesions were evident in 3 days at 20° C. Acervuli of the sporulating strain and sclerotia of the mycelial strain began to form within a week.

As previous inoculations failed to induce lesions on the stem, several variations in method of inoculation were made. Plants were left in the moist chamber for 48, 60, and 72 hours instead of the usual 24 hours. They were covered with glass jars to maintain a high relative humidity around them for 7 to 10 days after the inoculation period. The stem was wrapped in a moist cloth following inoculation and the cloth was kept moist by frequent spraying. None of these methods produced stem lesions. When needle wounds on stems were made just before or immediately after the spore suspension was applied, straw-colored lesions developed around the wounds, and, if the stem was kept continuously wet, the lesions occasionally developed up to 6 mm. in length. The fungus was successfully isolated from the diseased tissue. Thus it was shown that the organism developed rather feebly but only as a wound parasite on the stem.

For comparison, parallel inoculations with pycnidiospores of *Mycosphaerella pinodes* were made. Numerous brown lesions appeared both on stems and leaves. Except for the absence of sclerotia and acervuli, these lesions were comparable or identical to those observed in the field from which *Colletotrichum pisi* was isolated. The lesions were usually so numerous that they nearly covered the stem and leaf surfaces when the concentration of spores in the suspension was about the same as that used with *C. pisi*. The inoculated plants usually were killed in 10 or 14 days, although the spore concentration in the inoculum was considerably reduced.

PENETRATION

Penetration of the host by *Colletotrichum pisi* has been described and illustrated by Jones and Vaughan (8). In view of the irregularity of infection secured in inoculation experiments, the extent of penetration in leaves and stems was studied. At different intervals after inoculation thin layers of tissue were removed, cleared with lactophenol, and stained with cotton blue. A majority of the spores had germinated, and appressoria were abundant at 24 hours. The percentage of infection pegs from appressoria which had pierced the cuticle of the leaf tissue was very low. Moreover, penetration was never observed in stem tissues, although numerous appressoria formed on the surface of stems. This is in accord with the fact that infection of unwounded pea stems was never attained in the inoculation experiments.

INTERACTION OF MYCOSPHAERELLA PINODES AND COLLETOTRICHUM PISI

In view of the fact that anthracnose lesions were found commonly on stems in nature, it was postulated that they resulted when the organism followed a primary invader. Since *M. pinodes* was isolated most commonly along with *C. pisi* from such lesions, the interaction of these 2 fungi in infection was studied in the greenhouse. Pycnidiospores of *M. pinodes* and conidia of *C. pisi* were suspended together in water and the suspension applied with a brush to stems of pea plants in a moist chamber. Brown lesions typical of *M. pinodes* resulted. From these brown lesions, however, *C. pisi* was also reisolated readily. The same results were secured when the spores of *M. pinodes* were applied first and those of *C. pisi* 3 days later. Thus it was demonstrated that *C. pisi* may establish itself readily in pea stems when it infects at the same time as *M. pinodes* or following it. Under the conditions of these experiments, the rate of disease development was very rapid. The symptoms were more typical of *M. pinodes* alone, and sclerotia and setae of *C. pisi* did not develop, as is the case in the field. This difference is believed to be accounted for by the rapidity with which *M. pinodes* killed plants.

SEED TRANSMISSION

Since seed transmission of *Colletotrichum pisi* had not been previously demonstrated several hundred seeds from each of several commercial lots were plated on potato-dextrose agar. No *C. pisi* was found. Twelve hundred seeds from badly diseased pods were removed carefully and plated; from 5 of these, colonies of *C. pisi* developed. About 200 artificially inoculated seeds were plated at 6 and 10 months after inoculation. All of them yielded *C. pisi*. This showed that the organism penetrated the seed only occasionally in nature, but once it infected the seed it might live for a considerable period, perhaps a year or more, without losing its vitality. It is also possible that sclerotia are carried with the seed as contaminants.

DISCUSSION

In Wisconsin the prevalence of pea anthracnose in the field during certain

seasons has often given the impression that the disease is a serious one. The isolation and inoculation experiments reported in this study show, however, that although *Colletotrichum pisi* may occur as an independent pathogen on leaves and pods, it is impotent on stems. Its common association with *Ascochyta spp.* on stems indicates that one or another of these fungi commonly serves as the primary aggressor in lesions which are commonly interpreted as "anthracnose lesions" because sclerotia and setae of *C. pisi* are more conspicuous than the fruiting bodies of *Ascochyta.* This is particularly true with stem lesions which can not, apparently, be initiated by *C. pisi.*

In its rôle as a secondary parasite *C. pisi* is not unlike many other anthracnose organisms. *Colletotrichum gloeosporioides* Penz. commonly occurs on citrus without becoming an active parasite (3, 10, 15). *C. falcatum* Went establishes itself only after wounds (19). The cotton anthracnose organism (*Glomerella gossypii* (South.) Edg.) produces more conspicuous, more numerous, and more typical lesions when associated with the angular-leaf-spot organism (*Phytomonas malvacearum* (EFS) Bergey *et al.*) (18). *Colletotrichum circinans* (Berk.) Voglino develops on senescent lower leaves of onion but not on vigorously growing leaves (16). *C. coffeanum* Noack is found on living as well as die-back twigs of coffee, but the primary predisposing factor for the die-back is premature leaf fall due to *Hemileia vastatrix* B. et Br. (9). Shear and Wood (14) noted in their investigation that leaves and fruits of many plants collected in the field or in the greenhouse developed anthracnose (*Glomerella cingulata* (Atk.) S. & S.) when placed in a moist chamber. Roberts (13) isolated *G. cingulata* commonly from apple stem cankers caused by other pathogens.

It would appear from these studies that anthracnose is not so destructive a disease of pea as it may commonly appear upon casual observation. In epidemics of the disease, stem lesions are the most striking evidence of severe damage. In view of the fact that the organism appears to be incapable of acting as an independent parasite on stems, at least without the aid of wounds, and in view of the fact that *Ascochyta spp.* were isolated regularly from lesions in which *Colletotrichum pisi* was present, its importance has undoubtedly been overestimated. This overestimation is the more likely because, in the case of *Mycosphaerella pinodes* particularly as a forerunner, *C. pisi* fruits and produces sclerotia readily on lesions, while the former produces pycnidia relatively sparsely. It is only upon plating lesions on agar that the presence of the 2 organisms is revealed with certainty. It is also quite possible that other foliage pathogens of pea may act as predisposing agents for the anthracnose organism.

SUMMARY

Colletotrichum pisi has been interpreted occasionally as a serious pathogen of pea in Wisconsin.

Extensive isolation experiments showed that in a majority of cases *Ascochyta spp.* were isolated along with *C. pisi* from so-called typical anthracnose lesions.

In greenhouse inoculation experiments no infection of stems was secured except for feeble development around needle wounds, while infection of leaves was confined to those that were mature or senescent. Detached pods were infected when inoculated in a moist chamber.

When stems were inoculated with a mixture of spores of *Mycosphaerella pinodes* and *C. pisi*, or when those of the latter were applied several days after inoculation with the former, lesions resulted which were similar to young "anthracnose" lesions secured in the field.

Studies of *C. pisi* on leaves and stems showed that although conidia germinated readily and appressoria were formed, only a small percentage of the latter produced penetrating hyphae on leaves, while no penetration was observed on stems.

It is concluded that, in the main, *C. pisi* acts as a secondary pathogen in the field, particularly insofar as the conspicuous stem lesions are concerned.

Five out of 1200 seeds from naturally infected pods yielded *C. pisi*, showing that the latter may be seed-borne.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

LITERATURE CITED

1. ANTONOVA, S. P. [Anthracnose of pea.] (Abstr.) Bull. Plant Protect. [Leningrad] **1940**: 133–136. 1940.
2. BISBY, G. R., and I. L. CONNER. Plant diseases new to Manitoba. Sci. Agr. **8**: 456–458. 1928.
3. CLAUSEN, R. R. A new fungus concerned in wither tip of varieties of *Citrus medica*. Phytopath. **2**: 217–234. 1912.
4. Connecticut Agricultural Experiment Station. Personal service in plant diseases. *In* Conn. Agr. Exp. Stat. Bull. 337: 459–460. 1932.
5. DRAYTON, I. L. A summary of the prevalence of plant diseases in the Dominion of Canada. Canada Dept. Agr. Div. Bot. Bull. (N. S.) 71. 1926.
6. HEMMI, T. Nachtrage zur Kenntnis der Gloeosporien. Hokkaido Imp. Univ., Jour. Faculty Agr., **9**: 305–346. 1921.
7. JONES, F. R., and M. B. LINFORD. Pea disease survey in Wisconsin. Wis. Agr. Exp. Stat. Res. Bull. 64. 1925.
8. ———, and R. E. VAUGHAN. Anthracnose of the garden pea. Phytopath. **11**: 500–503. 1921.
9. MAYNE, W. W. Annual report of the coffee scientific officer, 1934–35. Mysore Coffee Exp. Stat. Bull. 13. 1935.
10. MCALPINE, D. Fungous diseases of citrus trees in Australia and their treatment. 1889.
11. PATOUILLARD, N., and G. DE LAGERHEIM. Champignons de l'Equator. Soc. Mycol. de France, Bull. **7**: 158–184. 1891.
12. RAMSAY, G. B. Anthracnose of pea in Louisiana. U. S. Dept. Agr., Plant Dis. Reptr. **27**: 255. 1943.
13. ROBERTS, J. W. The sources of apple bitter-rot infections. U. S. Dept. Agr. Bull. 684. 1918.
14. SHEAR, G. L., and A. K. WOOD. Studies of fungous parasites belonging to the genus Glomerella. U. S. Dept. Agr. Bur. Plant Indus. Bull. 252. 1913.
15. SMITH, R. E. Wither-tip. Calif. Cult. **37**: 76–77. 1911.
16. WALKER, J. C. Onion smudge. Jour. Agr. Res. [U.S.] **20**: 685–722. 1921.
17. ———, and W. W. HARE. Pea diseases in Wisconsin in 1942. Wis. Agr. Exp. Stat. Res. Bull. 145. 1943.
18. WEINDLING, R., and P. L. MILLER. The relation of *Bacterium malvacearum* to anthracnose boll rot of cotton. Phytopath. **31**: 24. 1941.
19. WENT, F. A. F. C. Het Rood Snot. (Review) Arch. Java Suikerindus. **1**: 265–282. 1893.

PHYTOPATHOLOGICAL NOTES

Physiologic Specialization in Helminthosporium gramineum Rabh.—Physiologic specialization in the fungus that causes barley stripe has been reported by several workers, but generally the specialization has not been clear-cut. Atlas has been reported resistant to stripe in Wisconsin,[1] although it is susceptible in California.[2] Recently a stripe culture pathogenic to Atlas was isolated from a naturally infected F_1 plant in the greenhouse at Madison. This culture, designated GHA2, was tested on Oderbrucker (C.I. 4666) and Colsess IV (C.I. 5979), and on Atlas (C.I. 4118) from three sources.

TABLE 1.—*Reaction of barley varieties when inoculated with different cultures of Helminthosporium gramineum Rabh., Madison, Wisconsin, 1944*

Culture	Trial[a] number	Stripe reaction[b] of barley varieties				
		Wisconsin Atlas	California Atlas	C.I. 4118 Atlas	Oderbrucker Wis. Ped. 5–1	Colsess IV
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
C–1	1	0	0	.....	82	.....
	2	9	0	0	91	90
	3	3	0	0	85	91
	4	0	0	.....	86	80
	Ave.	3	0	0	86	87
GHA2	1	50	7	.....	0	.....
	2	77	71	85	0	68
	3	89	92	98	0	96
	4	72	64	.....	3	77
	Ave.	72	59	92	1	80
45–2	2	5	0	0	100	100
	4	0	0	0	93	100
	Ave.	3	0	0	97	100

[a] Trials were started on the following dates: 1 on Mar. 10, 1944, 2 on Oct. 13, 1944, 3 on Nov. 21, 1944, and 4 on Dec. 8, 1944.

[b] The reaction in each trial is the average of 2 replications.

The Wisconsin Atlas had been carried in the breeding program for a number of years, the California Atlas was obtained from C. A. Suneson at Davis, and the C.I. 4118 Atlas was in the C.I. collection grown by R. G. Shands at Madison in 1942. The reactions of these varieties to 3 stripe cultures are shown in table 1. Cultures C–1 and 45–2 have been used for several years in varietal trials, and are of Wisconsin origin.

The Atlas from the 3 sources reacted similarly, although the one from Wisconsin was slightly more susceptible. Atlas was resistant to C–1 and 45–2, but susceptible to GHA2. Oderbrucker was susceptible to C–1 and 45–2, but highly resistant to GHA2. Colsess was susceptible to all 3 cul-

[1] Shands, H. L., and D. C. Arny. Stripe reaction of spring barley varieties. Phytopath. **34**: 572–585. 1944.

[2] Suneson, C. A., and Sylvia C. Santoni. Barley varieties resistant to stripe. (Note.) Jour. Amer. Soc. Agron. **35**: 736–737. 1943.

tures. These results show that stripe culture GHA2 is pathogenically distinct from cultures C–1 and 45–2.—D. C. ARNY, Departments of Agronomy and Plant Pathology, University of Wisconsin, Madison, Wisconsin.

Nematode Infection of Croft Easter Lilies.—A preliminary survey of greenhouses in the State of Washington during April, 1944, showed a rather general infection of Croft Easter lilies by the bud and leaf nematode, *Aphelenchoides olesistus* (Ritzema Bos) Steiner and Buhrer. Field examinations of lily plantings during the summer of 1944 also showed the infection to be rather general throughout areas producing this crop.

Infected plants (greenhouse forced and field grown) have strongly undercurled leaves which become bronzy and later turn brown and die. This condition had been previously observed by McWhorter[1] and named "dieback." Experience to date indicates that this symptom can be used in the field for identifying infected plants.

Microscopical examination of 155 lily plants with dieback revealed heavy infections of this bud and leaf nematode in all bronzed leaves. No other type of foliage carried infection.

Dieback plants were dug during September, 1944, and the bulbs replanted without treatment in October on soil that had never produced lilies, but had been used for many years as a chicken run. Examination of these bulbs at regular intervals following planting shows a constant infection of *Aphelenchoides olesistus* in all stages of development, between the growing tip (next year's foliage and bloom) and its surrounding scales. The number of nematodes per bulb has varied from 3 to 900.

These observations confirm the fact that dieback of the Croft Easter lily in Washington is caused by an infection of the bud and leaf nematode, *Aphelenchoides olesistus,* and that this infection is carried in the propagation stock. Experiments on phases of life history and possible control of this nematode are in progress.—WILBUR D. COURTNEY, U. S. Dept. Agr., Division of Nematology, Western Wash. Experiment Station, Puyallup, Washington.

Inoculations of the Evergreen Species of Prunus (Laurocerasus) with Tranzschelia pruni-spinosae.—The literature does not list any evergreen species of *Prunus* as hosts of the rust fungus, *Tranzschelia pruni-spinosae* (Pers.) Diet. Of the dozen or more evergreen species those available for inoculation were: *Prunus caroliniana* Ait., *P. ilicifolia* Walp., *P. ilicifolia* var. *integrifolia* Sudw. (*P. lyoni* Sorey), *P. Laurocerasus* Linn., *P. lusitanica* Linn., and *P. schipkaensis* Spaeth.

Prunus caroliniana and *P. ilicifolia* were infected by artificial inoculation with urediospores of *Tranzschelia pruni-spinosae discolor* Dunegan from peach and *P. pumila.* No evidence of infection developed on the other species.

[1] McWhorter, Frank P., S. L. Emsweller, and Philip Brierley. Suggestions for growing Easter lily bulbs in the Pacific Northwest. Ore. State Exp. Sta. Cir. 339. 1944.

The methods used were as follows: The foliage of the plant to be inoculated was sprinkled with water, and the inoculum on the rust-infected leaves was placed in contact with the healthy leaves. The inoculated plants or plant parts were enclosed in a moist chamber or in a cellophane bag. In other experiments the plant was enclosed in a cheesecloth chamber (Fig. 1,

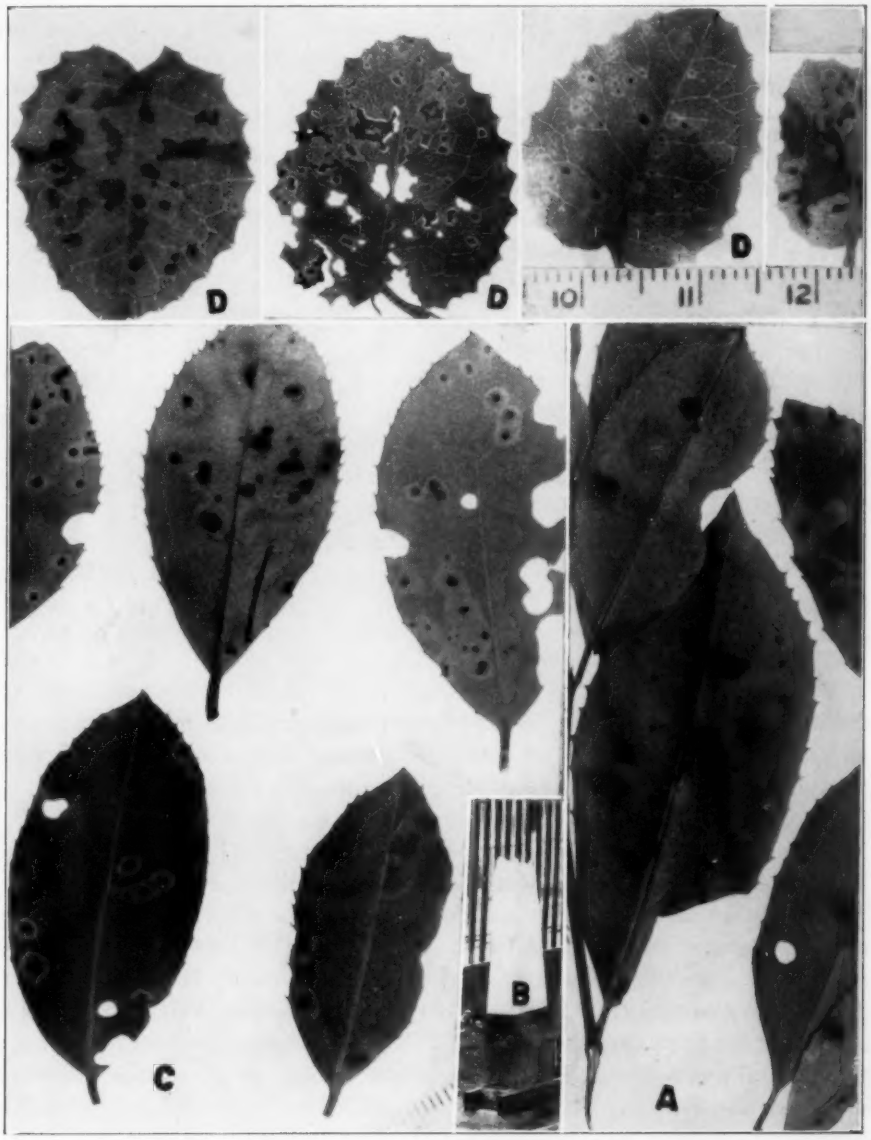


FIG. 1. Rust infection on certain evergreen species of *Prunus:* A, natural infection on *P. caroliniana;* B, a cheesecloth chamber used in the inoculation experiments; C, results of artificial inoculations on *P. caroliniana* (note millimeter scale near bottom). The infected areas had fallen out or were about to fall out. D, results of artificial inoculations on *P. ilicifolia.*

B) which was kept moist by a cheesecloth wick from an overhead vessel of water. In some other experiments the leaves on a detached twig were inoculated, and the stem was inserted in moist sand in a moist chamber. Satisfactory infection was secured by all methods.

Definite circular spots, 1–5 mm. in diameter (Fig. 1, C), developed on the leaves of *Prunus caroliniana* in about 3 weeks. The center of the spot was dark brown, surrounded by a greenish yellow zone. The tissues collapsed and a few urediospores of the fungus developed on these spots. The collapsed areas soon broke away from the healthy tissue, leaving a shot hole or, often, a ragged appearance to the leaf. The spots frequently coalesced.

Infection on *Prunus ilicifolia* and *P. ilicifolia* var. *integrifolia* (Fig. 1, D) was similar to that on *P. caroliniana*. There was at first a yellowing of the infection points which gradually darkened and became brownish spots 2–3 mm. in diameter. The brownish areas did not enlarge but became surrounded by a yellowish-green zone. There was a tendency for the infected tissue (the spots) to break away from the healthy tissue, but the shot holes were not so frequent as in *P. caroliniana*. Fruiting of the fungus was sparse but some typical urediospores formed on some of the spots. Infection usually occurred on the younger leaves.

It seems apparent, in both species, that the urediospores of the rust fungus must come from some more susceptible species of *Prunus* and that the fungus probably can not perpetuate itself on these hosts.

Smith and Cochran,[1] in their description of rust on the species of *Prunus* native to California, listed *Prunus ilicifolia* as not attacked by the rust fungus, and natural infection has yet to be observed.—CLAYTON O. SMITH, University of California Citrus Experiment Station, Riverside, California.

Polyporus Versicolor on Asiatic Chestnut.—Asiatic chestnut trees for ornamental and orchard purposes are now listed for sale by a few seed houses and nurseries. They may become of considerable economic importance in the near future. Thus the susceptibility of these trees to pathogenic fungi found in America is a matter of general interest to plant pathologists.

Some Asiatic chestnut trees have been growing since 1931 at the Syracuse Experiment Station of The New York State College of Forestry, Syracuse, New York, in an area where native chestnut once grew. While making routine observations of the trees in the autumn of 1944, sporophores of *Polyporus versicolor* Fries were discovered on one small tree. The sporophores had emerged from live bark covering a somewhat enlarged localized area on each of two branches (Fig. 1). Rabbits had gnawed the base of the tree several years previous and these wounds may have been the source of entry for the fungus. The typical spongy white rot associated with this fungus was present through the heartwood of the trunk and branches.

Polyporus versicolor, commonly associated with the decay of sapwood, is known to cause decay of the heartwood in living catalpa, especially pollarded

[1] Smith, Clayton O., and L. C. Cochran. Rust on the California native prune. Phytopath. 29: 645–646. 1939.

catalpa commonly used for ornamental purposes. The fungus has also been reported to cause heartrot of black cherry, London plane, apple, pear, and plum. Destruction of the heartwood weakens the affected trees and makes them especially susceptible to wind, snow, and ice damage. Consequently it is important for those who care for orchard and ornamental trees to be

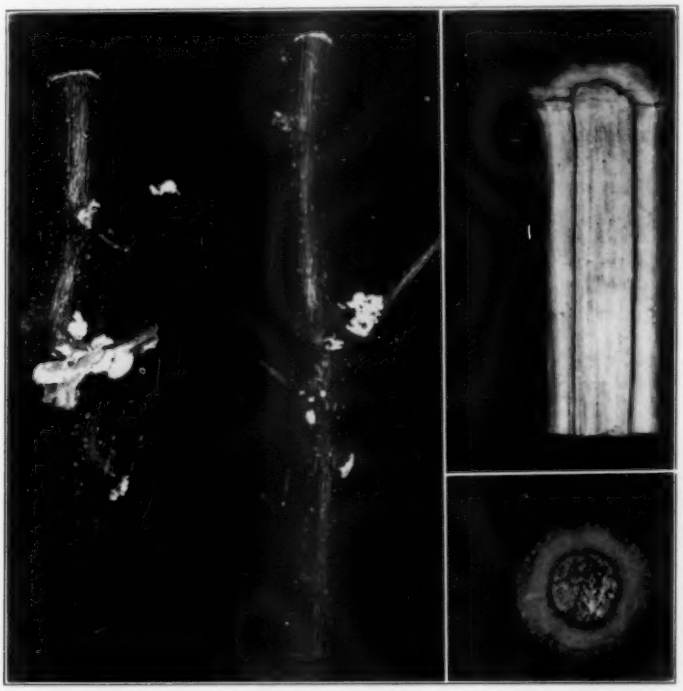


FIG 1. *Polyporus versicolor* causing decay of living Asiatic chestnut.

familiar with the fungi that cause heartrot, in order that proper bracing and cabling can be done before breakage occurs in the infected trees. Thus the association of *Polyporus versicolor* with heartrot of Asiatic chestnut is of interest to tree experts as well as plant pathologists.—RAY R. HIRT and J. L. LOWE. The New York State College of Forestry, Syracuse, N. Y.